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STRAWBERRY VIRUS DISSEMINATION IN ARKANSAS

Joseph P. Fulton

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SUMMARY

Indexing of commercial strawberry plantings has indicated that most are infected with a complex of viruses—various mixtures of mottle virus, vein-banding virus, crinkle virus, latent-A virus, and virus C. When indicator plants were exposed among infected plants, and virus-free plantings established near infected plantings were sample-indexed, only mottle and vein-banding viruses regularly spread, even after exposure of as long as 3 years. Mottle virus spread over an extended period, April through November. Rate of spread and build-up were much slower than anticipated. Studies on a stock of the variety Blakemore with June (genetic) yellows, grown locally since 1931, indicated a different virus situation than other commercial plantings. Mottle was the only virus commonly present, and in much lower amounts than anticipated. A large planting of this local stock was located that was apparently virus-free.

Introduction.—Strawberry plant certification programs, established in many areas of the United States, are based largely upon the release of virus-free stocks. Such programs were established after existing stocks were shown to be infected with one or more viruses. Since strawberries are vegetatively propagated and planting stocks are often shipped throughout the United States, the demonstration of virus in commercial stocks indicates little about the dissemination of viruses in a particular area. Furthermore, such information is not readily obtained from routine observations because the viruses generally occurring in eastern United States produce no obvious symptoms in most commercial strawberry varieties. In England, information on dissemination has been obtained with indicator plants by exposing such plants in field situations (15) or sample-indexing various types of field plantings (14). Widespread use of virus-free plants following the establishment of a strawberry certification program in Arkansas (8) presented an excellent opportunity for study of virus spread and infection. Such information is needed for determining the efficiency of strawberry certification in controlling the viruses.

Methods.—The indicator plant generally used in this study was a selection of the East Malling clone of *Fragaria vesca* L. Selection (EMK) was made from East Malling clone plants subjected to prolonged heat treatments. Repeated checking indicated that the selection was free of latent-A virus (5), which is generally present in the East Malling clone. The clone EMK has been used extensively by the author for several years because it reacts to the strawberry viruses very similarly to the regular East Malling clone yet does not contain latent-A virus. In checking the identity of viruses obtained, use was also made of several other clones of *F. vesca*, including the usual East Malling clone (9), UC-1 (6), and the runnerless Alpine seedling of *F. vesca* (6).

The designation of the various viruses follows that used by Frazier and Posnette (6). In most cases, virus determination was on the basis of host reactions. Virus C causes the host reaction referred to by McGrew (10, 11) as latent-C virus. In this study, comparisons were made with known isolates of various strawberry viruses—kindly supplied by Frazier (mottle, vein-banding, and mild yellow edge) and McGrew (virus C and latent-A). Herein "complex" refers to symptoms in *F. vesca* consisting of severe stunting, yellowing, mottling, distortion of leaves, and proliferation of the crown. These host reactions have been referred to by Demaree and Marcus (4) as being caused by a mixture of their type I and type II viruses. The symptoms can be obtained with virus mixtures containing various combinations of mottle, virus C, vein-banding, crinkle, and latent-A.

To determine the season of virus spread, groups of 50 plants of EMK were exposed for monthly intervals in a planting of Blakemore strawberries infected with several viruses. At the end of each month, the exposed plants were freed of aphids and held long enough for virus symptoms to develop.

To measure spread into virus-free plantings, leaves were collected at random and indexed by means of the excised-leaf grafting technique described by Bringhurst and Voth (1). Usually, a single leaf was grafted to a single indicator plant. In a few cases, when collections were made in isolated plantings of virus-free plants and little or no virus infection was anticipated, several leaves were grafted to a single indicator. It has been shown by Fulton (7) and Cropley (2) that results are sometimes erratic with the excised-leaf technique, though Miller (12) reported reliable results. Since the author believes that leaf grafts do not always assure virus transmission, results from this technique are interpreted with caution. In addition to the above collections, collections were usually made in nearby plantings known to be infected with virus. These were handled in a similar manner and served as a check on the technique as well as a check on the presence of virus in the infected source.

All commercial strawberries investigated were of the Blakemore Variety. No insecticides for the control of aphids were used in any of the plantings discussed herein.

Seasonal virus spread.—Table 1 shows the number of plants infected with mottle in 50-plant samples exposed for monthly intervals. Several virus mixtures, including mottle, virus C, crinkle, and latent-A, were present in the infected Blakemore plants. Only mottle appeared in the indicator plants, as shown in Table 1.

The results indicate that mottle virus can spread during most of the year, with peaks of dissemination in the spring and fall. Studies were not continued

Table 1.—Monthly spread of mottle virus into 50 plant samples exposed in strawberry fields containing plants infected with mottle, virus C, crinkle, and latent-A viruses. No spread of virus was found in December, January, February, and March.

	1957	1958	1959
April	–	1	19
May	–	10	11
June	7	2	
July	3	1	
August	0	1	
September	1	0	
October	0	9	
November	0	14	

long enough to exclude the possibility of some dissemination during mild periods in the winter. Since the indicator plants were set in the rows of infected Blakemore strawberries, it is possible that both crawling aphids and winged forms were responsible for virus dissemination.

Infection of virus-free plantings.—*Isolated plantings.*—Arkansas certification regulations require that virus-free plants to be sold under a Blue Tag label must be isolated at least 1 mile from other strawberries. No sample indexing of certified plantings is required. To check possible virus spread, however, many leaf collections were made in such plantings that had been in the field for 6–10 months. To date, no virus infection has been detected. During 1957 and 1958, for example, no virus was demonstrated in 637 leaves from such plantings grafted to indicator plants.

Two isolated plantings exposed for longer periods were checked. From a planting near Fayetteville, Arkansas, no virus was demonstrated in 123 plants indexed after 24 months in the field. From a planting near Augusta, Arkansas, no virus was demonstrated in 47 plants indexed after 24 months in the field.

Unisolated plantings.—Leaf collections were made in several locations where virus-free plants were exposed for various periods to nearby sources of virus. In most cases, such plantings had been established as a few rows on the border or in the center of an infected planting. Some collections were also made in virus-free plantings a short distance from infected plants. Results of this indexing are shown in Table 2.

At each of the 3 locations, the apparent incidence of virus in the virus-free plantings was much less than had been anticipated. It also appears that only nonpersistent types of virus moved in the areas under study, with mottle spreading the most frequently. A leaf-curl virus, similar to a mild type of vein-banding, was encountered at location A. When certain of the plants from this location were heat-treated (13), it became apparent that both mottle and leaf curl were present. Very faint vein-banding symptoms occurred following heat treatment. When mottle was added to such a plant, the more severe symptoms typical of the indexed field plants developed. No specimens appeared to have only mottle. Definitive work was not conducted on all positive indicator plants, so it is impossible to say whether vein-banding virus occurred singly in any. For that reason all are recorded as a vein-banding and mottle mixture.

At location C, a single case was recorded of the dissemination of viruses other than the nonpersistent viruses. In this case, the indicator plant developed symptoms typical of virus C plus mottle. Very little movement of any of the viruses was recorded at this location.

The results from locations A and B indicate that, though nonpersistent viruses disseminated into ad-

Table 2.—Virus dissemination at 3 locations from infected sources into nearby virus-free plantings.

Location & exposure [b]	Total grafts	Positive for virus [a]			
		Complex	M	VB+M	C
A) Infected source plants	57	27	0	9	1
Virus-free plants, 8 rows in above:					
22 mo.	77	0	0	16	0
36 mo.	13	0	0	5	0
Virus-free plants (7 A.), ¼ mi. NE of above:					
10 mo.	87	0	0	3	0
24 mo.	51	0	0	2	0
B) Infected source plants	35	19	6	0	0
Virus-free plants:					
12 mo.	122	0	28	0	0
24 mo.	24	0	10	0	0
36 mo.	24	0	14	0	0
C) Infected source plants	63	47	3	0	4
Virus-free plants (border rows):					
12 mo.	21	0	0	0	0
24 mo.	71	1	1	0	0
30 mo.	75	0	1	0	0
36 mo.	152	0	1	0	0
Virus-free plants (4 A.), ¼ mi. S of above:					
24 mo.	34	0	0	0	0

[a] M = mottle; VB = vein-banding; C = virus C.

[b] A) near Siloam Springs, Ark.; infected source, 5-A. planting; B) near Fayetteville, Ark.; both infected source and virus-free plants occur as small, interspersed plots; C) near Bald Knob, Ark.; infected source, 4-A. planting.

joining virus-free plantings, they did not build up rapidly within the new plantings.

Viruses in local stocks of Blakemore.—In most areas of Arkansas and southern United States, the original stock of Blakemore has been generally replaced by the "yellows-free" strain (3). Growers in the Marshall (Arkansas) area, however, have continued to use the original stock even though June (genetic) yellows appears in practically all plants during periods of rapid growth. Five locations in the Marshall area were indexed in the same manner as the other locations. Since the virus disease situation in all plantings of the original stock of Blakemore appears to be somewhat different from that in other commercial stocks of Blakemore, results from this area are reported separately (Table 3). Location G served as a check for this area since plants were from an outside source. Indexing indicated that this outside stock of plants was infected with viruses in amounts similar to those in commercial stocks in other locations. In the original stock of Blakemore, however, mottle was the most common virus. The movement of virus C into this stock is apparently a rarity, as indicated by the single example at location F. The results at location D and E indicate that virus-free plantings of older stocks do occur in this area.

Aphid vectors.—Strawberry aphids have been observed regularly at the location mentioned herein. They were collected with ease during May and June, and again in October. In May and June of 1959, collections for identification were made at locations A, B, C, F, and H. All were identified as *Pentratrichopus minor* (Forbes). (The author gratefully acknowledges identification by Dr. G. A. Schaefers, New York Agricultural Experiment Station.)

Discussion.—The results indicate that a variety of strawberry viruses have been introduced into Arkansas. This mixture of viruses is similar to that reported from eastern and northern United States and, except for mild yellow-edge virus, from western United States. Only a part of this introduced mixture of viruses is regularly disseminated within the areas studied: the nonpersistent mottle and vein-banding viruses. Rorie (16) has suggested that the reason for such selective virus movement may be due to the species of aphid which occurs in this area. He (16) has further shown that *P. minor* is a relatively efficient vector of mottle under experimental conditions. The slow infestation of plantings with mottle or vein-banding at locations A and B indicates that this aphid may not be an efficient vector under field conditions. Furthermore, the relatively low infestations of mottle in plantings of the original stock of Blakemore in the Marshall area likewise suggest vector inefficiency. Reasons for the negligible virus dissemination, even though the vector is present at location C, are not immediately apparent. Further studies on virus-vector relationships and vector build-up and movement in Arkansas may reveal the reasons.

Studies such as those presented here are limited by year-to-year fluctuations in amounts of virus dissemination. In this particular study, however, the results conform so closely to those obtained in indexing the original stock of Blakemore, which has been increased locally since 1931, that these results seem indicative of the actual long-term situation. This assumes, of course, that the original stock of Blakemore was free of virus when introduced. This seems the most logical explanation for the fact that large plantings of the original stock of Blakemore are apparently still free of virus.

Table 3.—Sample indexing in strawberry plantings near Marshall, Arkansas.

Type of planting	Total grafts	Positive for virus		
		Complex	M	C
D) Local stock (12 A.)	133	0	0	0
E) Virus-free stock (1 A.) in planting D, 24 mo. exposure	91	0	0	0
F) Local stock (8 A.)	55	0	7	1
G) Commercial, yellows-free stock (4 A.)	17	11	0	0
H) Local stock (10 A.)	57	0	28	0

The rather slow virus dissemination and the low build-up of virus following introduction are often attributed to effective aphid-control programs. But aphid control was not practiced in any of the plantings discussed, and one would certainly question the wisdom of recommending such programs in the area. At least such vector-control programs would have to be very carefully evaluated from the standpoint of preventing virus dissemination before they could be recommended. Furthermore, reports of reduced virus dissemination following aphid control must be evaluated with caution unless sufficient checks are also reported to properly evaluate the actual effectiveness of the program in reducing virus dissemination.—Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.

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EXTRACELLULAR ENZYME AND TOXIN PRODUCTION BY FUSARIUM OXYSPORUM F. LINI

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SUMMARY

F. oxysporum f. *lini* produced abundant polygalacturonase and cellulase but little or no pectinmethylesterase. The flax wilt pathogen produced fusaric acid when cultured on a synthetic medium or on tissue from a wilt-susceptible variety, but not when cultured on tissue of wilt-resistant varieties. Fusaric acid induced severe wilting in flax cuttings, without differences between wilt-resistant and wilt-susceptible varieties.

Introduction.—The host-parasite relations of the Fusarium wilt disease of flax have been studied by various workers. Rüdiger (9) indicated that fungal enzymes are important in the flax wilt syndrome. Grossman (13) and Millikan (7) found that culture filtrates of the pathogen contained agents that induced wilt when applied to flax cuttings. The production of extracellular enzymes and toxins by *Fusarium oxysporum* f. *lini* (Bolley) Snyd. & Hans. was therefore investigated.

Methods.—The organism used was *F. oxysporum* f. *lini*, clone 294 (pathogenic single-spore isolate). The nutrient medium for preparing culture filtrates for enzyme assays was 5m*M* KH_2PO_4, 2m*M* KNO_3, 2m*M* $Ca(NO_3)_2$, 1m*M* $MgSO_4$, 0.5 ml Hoagland's minor elements (5), chelated Fe (0.3 mg Fe), 12.5 g sucrose, 12.5 g pectin, 3 g Bacto yeast extract, 3 g Bactopeptone, and distilled water to make 1000 ml. Wheat bran cultures (12) were also used in an attempt to increase pectinmethylesterase (PME) activity. The stationary cultures were grown 2 weeks at 25°C in 2500-ml flat-bottom culture flasks containing 500 ml medium. Then the culture medium was filtered through a Büchner funnel, centrifuged 10 min. at 2000 G, and passed through a bacteria-retaining filter (Millipore Corp). The clear filtrate was used immediately as the enzyme sample.

PME activity was measured by the continuous titration method of Kertesz (6). The reaction mixture consisted of 50 ml culture filtrate and 100 ml substrate solution. The substrate was a 1.5% solution of citrus pectin (practical grade, Eastman) adjusted to pH 4.5 during the reaction.

Polygalacturonase (PG) and cellulase (Cx) activities, determined by measuring viscosity loss in the substrate-enzyme mixture, were confirmed by chromatographic detection of the products of enzymatic hydrolysis. Sodium polypectate (1.2% product no. 24, Sunkist Growers, Ontario, California) buffered at pH 5.0 served as substrate for PG. The substrate for Cx was carboxymethylcellulose (1.2% CMC-70 M premium grade, Hercules Powder Co.) buffered at pH 5.0. In the viscosity determinations of PG and Cx, 2-ml culture filtrate were added to 4-ml substrate solution. Viscosity measurements were made in Ostwald-Fenske viscosimeters (size 200) at 30±0.1°C. The chromatographic procedure of Echandi et al (1) was used to analyze the products of pectolytic and cellulolytic hydrolysis. A mixture of 10 ml culture filtrate and 20 ml substrate solution was incubated at 30°C, and samples for chromatographic analysis were taken at 0, 2, 8, and 24 hours.

Results.—Regardless of the culture medium, PME activity of the culture filtrate was either very low or undetectable. In 8 of the 10 trials no PME activity was detected, and in 2 trials the activity was 0.03 mg methoxyl removed per ml culture filtrate in 30 minutes. A 0.02% preparation of commercial PME (Pectinol, Rohm and Haas Co.), used as a control, showed considerable activity.

When sterile culture filtrates were added to 1.2% sodium polypectate, the viscosity dropped very rapidly within 10 minutes (Fig. 1), indicating the presence of PG and/or pectindepolymerase. Culture filtrates heated 15 minutes at 95°C did not decrease the viscosity of the sodium polypectate. Chromatographic analysis of the products of enzymatic hydrolysis (employing sterile culture filtrates) established the presence of galacturonic acid after a minimum reaction time of 8 hours. Substrate, heat-inactivated enzyme-substrate mixture, and galacturonic acid were used as controls. Since galacturonic acid was released during the reaction it was concluded that the hydrolytic enzyme PG was present in the culture filtrate.

Culture filtrate from a medium containing sucrose and pectin as the carbon source caused rapid viscosity

loss in carboxymethylcellulose solutions, indicating the presence of cellulase (Fig. 1). Culture filtrates heated 20 minutes at 95°C did not decrease the viscosity of CMC solutions. Glucose and cellobiose were detected chromatographically after the enzyme-CMC mixture had incubated for 8 hours. Substrate, heat-inactivated enzyme-substrate mixture, cellobiose, and glucose were used as controls.

To determine the capacity of the flax wilt pathogen to produce fusaric acid, the fungus was grown 14 days at 25°C in stationary culture in modified Richard's nutrient solution (10) containing 50 g glucose and 3 g glycine per liter. The fusaric acid was isolated from the culture solution and identified by the chromatographic methods of Zähner (13). Chromatographic and phytotoxic properties were the same in naturally produced fusaric acid as in synthetic fusaric acid (obtained through the courtesy of Dr. S. Naef-Roth, Zurich).

When freshly detached flax shoots (8 in. tall) of 4 wilt-susceptible and 3 wilt-resistant varieties were allowed to take up synthetic fusaric acid (200 μg fusaric acid per g fresh weight of shoots) at 25°C before transfer to Hoagland's mineral solution (5), induced wilting of the shoots progressed as follows: loss of turgidity in upper part of stem, causing growing point to droop (12–24 hours after toxin absorption); severe wilting and shriveling of leaves in the region of the growing point; small black spots on the distal portion of many leaves, the affected leaves soon becoming wilted and shriveled; turgidity loss, softness and collapse in the lower and often extreme upper portions of the stem (24–36 hours); change from normal green to a grayish and yellowish green in the stem and proximal portions of leaves on the lower part of the shoot; softening and collapse with continued flaccidity in tissue of entire shoot (36–48 hours). These gross symptoms do not exactly parallel the natural symptoms of flax wilt, for, as Gäumann pointed out (2), fusaric acid never acts alone on the host but always in association with other products of fungal and host metabolism. The effect of absorbed fusaric acid on the 7 flax varieties demonstrated that the wilt-resistant varieties were not more fusaric-acid-resistant than were the wilt-susceptible varieties.

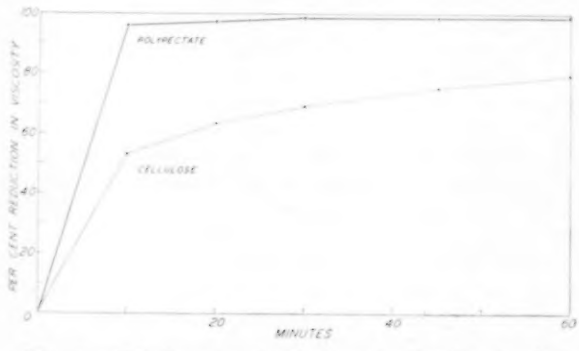

Fig. 1.—Relative rate of reduction in viscosity of sodium polypectate and carboxymethylcellulose by enzymes produced by *F. oxysporum* f. *lini*.

In another study the wilt pathogen was cultured on 1 susceptible (Punjab 47) and 2 resistant flax varieties (Dakotas 48-94 and 49-2), each in a separate flask. The entire plants (10 in. tall) were dried at 45°C and cut into 1-in. sections, and 100 g dried tissue was placed in a large culture flask. The flasks and their contents were then sterilized with propylene oxide (4). After the propylene oxide was removed by sterile aeration, 500 ml sterile distilled water was added. An active mycelial-spore suspension was transferred to each flask. After growth proceeded for 2 weeks at 25°C the media were filtered and the filtrates were analyzed for fusaric acid. The fungus appeared to grow with equal vigor on all 3 varieties, but fusaric acid was detected only in the culture filtrate of the susceptible variety.

Discussion.—It has been suggested that the extracellular enzymes and phytotoxins produced by *F. oxysporum* f. *lycopersici* (2, 8, 11, 12) and *F. oxysporum* f. *vasinfectum* (2, 12) function in the wilt syndrome of these vascular pathogens. PG and Cx production by *F. oxysporum* f. *lini* was consistent with that in previous studies with other Fusaria. *F. oxysporum* f. *lini*, however, produced little or no PME, which suggests a possible qualitative difference between this forma and other formae of *F. oxysporum*, which produce abundant PME.

When cultured on a synthetic medium or on tissue from a wilt-susceptible flax variety, the flax wilt pathogen produced fusaric acid. The inability of this fungus to produce fusaric acid when cultured on wilt-resistant flax varieties may indicate that flax wilt resistance to clone 294 is related to fusaric acid production by clone 294.

The *in vitro* production of extracellular enzymes and phytotoxin suggests that they may be implicated in the wilt disease of flax. Establishing their significance in the flax wilt syndrome, however, requires demonstration of their production in wilt-diseased tissue.—Department of Agronomy, University of California, Davis.

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THE HCN CONTENT OF FLAX IN RELATION TO FLAX WILT RESISTANCE

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SUMMARY

When grown in environments of controlled light intensity, photoperiod, temperature, and humidity, young flax tissue was very rich in cyanide, but the amount per unit fresh weight of shoots decreased with plant age. Increased light intensity increased tissue cyanide. Cyanide content of flax tissue decreased significantly during the dark period. It was postulated that free HCN may have been released into the physiological fluids at the growing point, reaching enzyme-inhibiting concentrations. Growth of *Fusarium oxysporum* f. *lini*, the flax wilt fungus, was strongly inhibited *in vitro* at 5m*M* (135 ppm) HCN. High cyanide content of flax varieties had no uniform correlation with resistance to either clone 294 or clone 33-1 of the wilt pathogen.

Introduction.—The outstanding feature of resistance to Fusarium wilt of flax is that the growth of *Fusarium oxysporum* f. *lini* (Bolley) Snyd. & Hans. is slowed in tissues of wilt-resistant varieties of *Linum usitatissimum* L., though the fungus penetrates resistant as well as susceptible varieties. Thus, the correlation of antifungal substances with wilt-resistant varieties appeared to be a direct approach to the chemical nature of flax wilt resistance. HCN, a strong metabolic inhibitor, is present in flax tissue in the form of a cyanogenic β-glucoside, linamarin. Since another forma of *Fusarium oxysporum* was shown (2, 18) to produce an extracellular β-glucosidase, it was suggested that the production of a β-glucosidase by the flax pathogen could release from flax tissue enough HCN to inhibit the growth of the invading organism. This paper reports on a test of this hypothesis.

It is well known that wilt resistance in flax varies with the environment. Therefore, an estimate was desired of the cyanide content of flax varieties grown in controlled environments. Herein the word *cyanide* refers to the bound cyanide of linamarin.

Literature review.—The cyanogenic glycosides occur throughout the plant kingdom. Some workers have focused particular interest on linamarin in flax (10, 11, 12, 16, 17). When hydrolyzed enzymatically, linamarin releases HCN, acetone, and glucose. In the flax plant linamarin appears to be the only source of HCN.

Working with the cyanide of flax seed, Lüdtke (10) reported that the cyanide content depended primarily on the flax variety, with little effect from fertilizer and soil treatments. More recently he demonstrated (11) that linamarin was formed in the green parts of flax plants, and that total cyanide in a plant increased up to the time of flowering. Lüdtke and Hahn (12) found practically no cyanide in the roots of flax, which agrees with the results of Reynolds (16), who found that flax roots contained about 20% as much cyanide as did the shoots (dry weight basis). In contrast, Timonin (17) reported the excretion of 25–35 mg HCN in 25 days from the roots of a single 30-day-old flax plant.

Reynolds (16) reported primarily on the cyanide content of flax in relation to the Fusarium wilt of flax. Conditions for flax growth and the storage of harvested samples were not uniformly controlled, but Reynolds concluded that at least part of flax wilt resistance may be attributed to the cyanogenic glucoside of flax. Testing the effect of HCN on the growth of *Fusarium lini* in sterile culture, he found (15, 16) that a concentration of 30m*M* HCN was required to inhibit fungus growth completely. Timonin's study (17) of one wilt-susceptible and one wilt-resistant variety supported Reynold's hypothesis that high HCN content may be related to wilt resistance.

Lüdtke and Hahn (12), working with 4 flax varieties susceptible and 4 resistant to *Colletotrichum lini* found no protection from high cyanide content. The varieties with the highest and lowest cyanide in the seedlings were completely destroyed. Since cyanide content was intermediate in the resistant varieties, it was thought not to be a criterion of resistance to *C. lini*.

Houston et al (7) demonstrated that flax varieties grown in sand culture watered with Hoagland's solution exhibited their normal susceptible or resistant wilt characteristics. Employing 7 flax varieties and 4 races of the wilt pathogen, Nair (14) found that the growth of the fungus was reduced in the shoots as well as the roots of the resistant varieties. Thus, factors for wilt resistance known to function in the roots also appeared to function in the shoots.

Methods and materials.—Several methods of determining the cyanide content of flax shoots were compared. The method finally chosen for the collection of HCN was a modification of the Hogg and Ahlgren method (5). The pyridine-pyrazolone procedure was used for detecting HCN (9), however, because specificity and sensitivity were greater than in the picric acid method employed by Hogg and Ahlgren. The

modified method was specific and simple, only a small amount of tissue was required, and the results were within experimental error of the results obtained by the most quantitative distillation method of Melville et al (13).

The procedure was to place 1.00±0.03 g of fresh-frozen flax tissue in a wide-mouth 250-ml Erlenmeyer flask and place within it a 4-dram vial containing 5 ml 1*N* NaOH and a small piece of folded filter paper to increase the surface area. The flask was stoppered immediately and maintained 24 hours at 25–28°C. Then the vial containing the NaOH, the folded filter paper and the absorbed HCN was removed and its contents diluted to 100 ml with deionized water. An aqueous mixture was then prepared by adding, in order, 70 ml deionized water, 10 ml 0.1*M* phosphate buffer (pH 6.8), 10 ml of the diluted alkaline HCN solution, and 10 ml 0.05 *N* HCl. A 10-ml aliquot of the resulting buffered HCN solution (pH 6.8) was used for color development by the method of Kruse and Mellon (9). The same procedure was followed in the determination of the standard curve after known amounts of HCN had been added to the NaOH in the vial. The resulting standard curve was linear from 0 to 7 μg HCN per 10-ml aliquot when absorbance at 620 mμ (Bausch & Lomb Spectronic Colorimeter) was plotted against concentration of HCN.

Several preliminary experiments on the cyanide content of flax shoots were compared before the described method was selected. The effects of tissue cytolysis and the addition of linamarase on the release of HCN from flax shoots was studied with the varieties Punjab 47 and Dakota 48-94. Both were grown in sand culture, watered daily with Hoagland's nutrient solution (4), and maintained at 20±1°C, a 12-hour photoperiod under 1000 ft-c (combined fluorescent and filament lamps), and 60–90% relative humidity (RH). Twenty-one days after emergence the plants were harvested, immediately frozen, and stored at –17°C in moisture-proof plastic bags. In this experiment 5 drops of chloroform per gram fresh weight of flax shoots were used for cytolysis. The enzyme preparation used was linamarase I, as described by Coop (1), 1 ml of 0.1% linamarase I being added per gram fresh weight of flax shoots. The remaining details for HCN determinations were as described above. The release of HCN from flax shoots by the distillation method of Melville et al (13) was also determined.

Another preliminary study determined the effects of reaction temperature and reaction time on the enzymatic release of HCN from flax shoots. The variety used was susceptible Punjab 47, grown under the conditions described above. Procedure was as described above but did not include the addition of chloroform or linamarase. In each of the preliminary experiments 4 replicate flasks were used for each treatment.

The growth of *F. oxysporum* f. *lini* in the presence of HCN was determined by growing the fungus in a chemically defined nutrient medium to which KCN had been added. The fungus used was "wild type" clone 294 (pathogenic single-spore isolate) (6). The chemically defined medium was 5m*M* KH_2PO_4, 2m*M* KNO_3, 2m*M* $Ca(NO_3)_2$, 1m*M* $MgSO_4$, 0.5 ml Hoagland's minor elements (4), chelated iron (0.3 mg Fe), 25 g sucrose, 3 g Bacto yeast extract, 3 g Bactopeptone, 1*N* HCl to lower pH to 4.0, and distilled water to make 1000 ml. At each HCN concentration the fungus was grown in 4 replicated 250-ml Erlenmeyer flasks, each containing 50 ml medium and the appropriate amount of KCN. The KCN was added to the cooled sterile medium via a bacteriological filter. The inoculum for each flask was 5 ml of a fragmented mycelium-spore suspension that had grown in shake culture in the same control medium for the preceding 24 hours. After receiving the KCN and the inoculum the flasks were stoppered tightly to prevent the escape of HCN and placed on a rotary shaker for 24 hours at 25°C. The mycelium in each flask was then filtered, dried at 45°C, and weighed.

The work of Knowles and Houston (6, 8) and Gautum (3) on the inheritance of flax wilt resistance formed the basis for selection of the flax varieties used: 4 wilt-susceptible—Punjab 47 (C.I. 1115), hereinafter designated S_1; Imperial (C.I. 1114), S_2; Dakota 48-90 (C.I. 1071), S_3; and Rio (C.I. 280), S_4; and 6 wilt-resistant—Redwood (C.I. 1130), R_1; Punjab 165-7 (C.I. 20), R_2; Redson (C.I. 970), R_3; Dakota 49-2 (a selection of Dakota), R_4; Redwing (C.I. 320), R_5; and Dakota 48-94 (a selection of Dakota), R_6.

In a study of the cyanide content of these 10 varieties, each variety was grown in sand culture in a 1-gal. glazed crock and watered daily with Hoagland's nutrient solution. The crocks were randomized in the growth chamber, which was held at 20±1°C, a 12-hour photoperiod under 1200 ft-c, and RH of 60–90%. The plants were harvested 21 days after emergence, near the middle of the light period. The shoots of each variety were divided into 5 samples, placed in moisture-proof plastic bags, frozen immediately, and stored at –17°C.

The effect of seedling age on the cyanide content of two varieties was determined by growing each variety in sand culture in 4 replicated flats maintained in a controlled growth chamber. The plants were watered daily with Hoagland's nutrient solution. Growing conditions were 20±1°C, 1200 ft-c, a 16-hour photoperiod, and 60–90% RH. Each flat was divided into quarters. One randomly chosen quarter of each flat was harvested at each of the 4 harvest dates. After harvest the shoots were frozen immediately and stored at –17°C.

The effect of light and dark periods and light intensities on the cyanide content of 2 varieties was determined in the controlled-environment chamber. Growing conditions were 20±1°C; 100, 1000, and 2500 ft-c; a 12-hour photoperiod, and 60–90% RH. The plants were grown in sand culture and watered daily with Hoagland's solution. Each variety was grown in 3 flats. One of the 3 flats of each variety was placed at each of the 3 light intensities. Each flat was divided into

Table 1.—Effects of cytolysis, linamarase, and distillation treatments on the release of HCN from flax shoots.

Treatment	ppm HCN (fresh weight) [a] Variety P47	Variety D-48-94
Frozen tissue	352	136
Frozen tissue + linamarase	362	132
Macerated frozen tissue + linamarase	354	136
Frozen tissue + chloroform	365	137
Frozen tissue + chloroform + linamarase	362	135
Distillation method (frozen tissue)	365	140

[a] Mean of 4 replications.

10 equal sections. When the seedlings were 21 days old, 5 of the 10 sections in each flat were harvested near the end of the light period. The remaining 5 sections of each flat were harvested 12 hours later, near the end of the dark period. Harvested plants were frozen immediately and stored at –17°C.

In an experiment to determine the amount of HCN released by the roots and/or shoots, 10 young flax plants from varieties S_1 and R_6 were transferred from sand culture to a large culture tube. With their roots immersed in aerated Hoagland's solution (diluted 1:4) the plants continued to develop normally. Light intensity during the 12-hour photoperiod varied between 100 and 300 ft-c. Temperature varied between 22 and

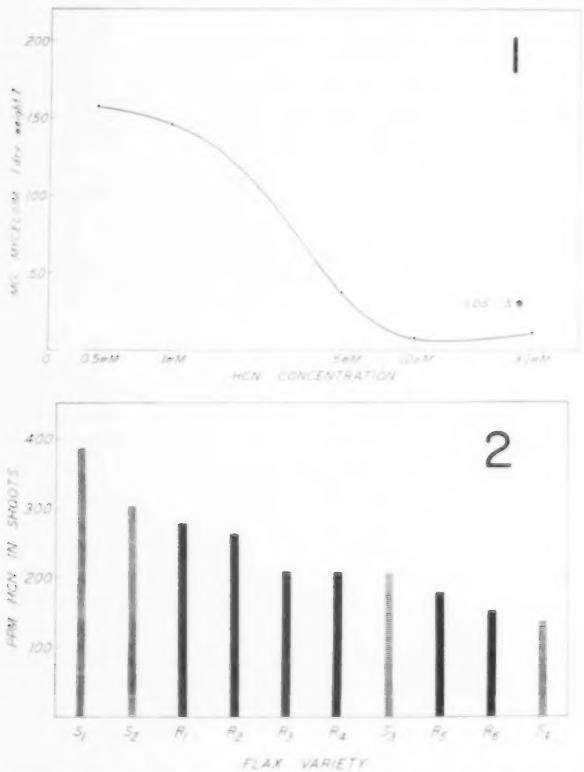

Fig. 1–2.—**1)** Growth of *Fusarium oxysporum* f. *lini* in 24 hours in liquid culture at 25°C in the presence of HCN. **2)** The HCN content of 4 wilt-susceptible and 6 wilt-resistant flax varieties grown in the same uniform environment (20°C, 1200 ft-c, 12-hour photoperiod).

Table 2.—Effects of reaction temperature and reaction time on the release of HCN from flax shoots.

Reaction temperature	ppm HCN (fresh weight) [a] 24 hours	36 hours
20°C	336	339
30°C	350	355
40°C	362	362

[a] Mean of 4 replications.

26°C. On the 21st day after germination all the air passing out of the culture tube for 24 hours was bubbled through 10 ml 1*N* NaOH. An aliquot of the NaOH trap was analyzed for HCN by the above method. The root solution was analyzed for HCN by the distillation method of Kruse and Mellon (9).

Standard statistical methods were used to analyze the experimental data.

Results.—Several methods of releasing and collecting HCN were investigated (Tables 1, 2). In all cases the flax shoots were frozen before HCN analysis. The frozen flax tissue gave maximum yields of HCN; cytolysis with chloroform was not necessary. Furthermore, the addition of linamarase to macerated or unmacerated flax tissue did not increase HCN release, indicating that sufficient enzyme was present to release the bound HCN. Melville's technique (13) of releasing HCN enzymatically, followed by a distillation method of collecting the released HCN, gave results very comparable to the modified method of Hogg and Ahlgren (frozen-tissue treatment). The experiment dealing with reaction temperature and time indicated that 95–100% of the tissue-bound HCN was released in 24 hours at 25–28°C (Table 2).

Growth of the wilt fungus in the presence of HCN was studied in tightly stoppered flasks to prevent the escape of highly volatile HCN. Fig. 1 shows the average growth of clone 294 of the pathogen in a chemically defined medium containing various concentrations of HCN. The pH of the medium before and after growth was 4.0±0.1. Growth of the fungus at concentrations of HCN greater than 1m*M* was strongly inhibited; at 5m*M* HCN it was only 23% of that of the control. Statistical treatment of the data indicated that LSD at the 5% level was 10.8. It was noteworthy that an average of 162 mg of mycelium was produced in each of the stoppered control flasks in 24 hours, compared with an average of 355 mg of mycelium produced in each of the well-aerated control flasks.

In an experiment in which the 10 flax varieties were grown in the same uniform environment the HCN contents of the varieties were found to vary from 137 to 386 ppm, fresh-weight basis (Fig. 2). The highest as well as the lowest values were for susceptible varieties. There was no significance at the 5% level between the means of the susceptible varieties compared with the means of the resistant varieties. Thus, no uniform correlation was found between cyanide content of flax varieties and resistance to clone 294 of the wilt pathogen.

The variation in cyanide content with increasing age of flax shoots was studied in 2 flax varieties grown in a controlled environment. The plants were harvested 5, 10, 17, and 20 days after emergence. Fig. 3 shows average cyanide content of the two varieties at harvest. In both varieties young seedlings were very rich in cyanide, and the amount of cyanide per unit fresh weight of shoots decreased as the plants grew older, at 10 days being about half that at 5 days. Decline then was more gradual. Statistical analysis of the data indicated significance at the 1% level for the varieties, harvest time, and variety–harvest-time interaction.

The effects of 3 light intensities and the length of light and dark periods on the cyanide content of shoots were studied in 2 varieties of flax. In both varieties the tissue cyanide increased as light intensity increased. Varietal effect and the variety–light-intensity interaction were both significant at the 5% level, but the effect of light intensity was not significant at that level. Under all conditions tested, cyanide content of shoots was consistently lower at the end of the dark period than at the end of the light period (Fig. 4). This diurnal variation in cyanide was proportionately greater in the variety with the most cyanide per unit weight. Statistical calculations indicated significance at the 1% level for the light period variations, and at the 5% level for the interactions involving variety × light period, light intensity × light period, and the 3-way interaction variety × light intensity × light period.

The release of large quantities of HCN from the roots of a wilt-resistant flax variety reported by Timonin (17) suggested a similar experiment. In addition to the liberation of HCN by the roots the release of free HCN into the air by the shoots of 2 flax varieties was tested by passing the air through a trap to absorb the cyanide. The methods used were extremely sensitive, but not a trace of HCN was found in either the root solution or the air stream of either variety. This single preliminary experiment was not replicated.

Discussion.—The diurnal variation in cyanide content of flax shoots is worthy of consideration. Though no work indicates the presence of free HCN in flax tissue, this paper shows less HCN detected at the end of the dark period than at the end of the light period, thus indicating that linamarin may be degraded during the dark period. If linamarin is broken down during the dark period and releases free HCN into the physiological fluids, it would not be unreasonable to expect the concentration to reach 1m*M* in flax-shoot tissue. HCN at that concentration can inhibit the action of certain metalo-porphyrin enzymes. It is thought that linamarin reaches its highest concentration near the terminal growing point, a localized region of many important physiological reactions. Thus, it is possible that HCN could enter into reactions near the growing point that play an important role in the physiology of the host.

It is interesting to speculate on why the growth of *F. oxysporum* f. *lini* is strongly inhibited in sterile liquid culture at 135 ppm HCN, whereas it attacks and develops in young flax plants whose shoots may contain more than 2000 ppm bound HCN. Linamarin, the HCN-containing glucoside of flax, may not be uniformly distributed throughout the shoot but localized in specific tissue. Roots are the initial point of attack of this soil pathogen, and Reynolds (16) and Lüdtke (11) showed that flax roots contain much less cyanide than flax shoots. Nothing is known about the intracellular localization of linamarin. Furthermore, the biochemistry of linamarin in healthy and diseased tissue remains obscure. Free HCN is possible in living flax tissue, but it has not been demonstrated, nor has the toxic effect of the intact linamarin molecule on the growth of the pathogen.

It is well known that a variety resistant to one clone of a pathogen may be susceptible to a different clone. When the pathogenicity of a second common clone of *F. oxysporum* f. *lini*, clone 33-1, was tested against the 10 varieties used in this study, 3 of the 10 varieties reacted differently. Flax varieties S_3 (Dakota 48-90) and S_4 (Rio), susceptible to clone 294, were very resistant to clone 33-1, but variety R_2 (Punjab 165-7), resistant to clone 294, was susceptible to clone 33-1 (3). The degree of wilting brought about by these 2 clones was 90–100% on the susceptible varieties, and only 0–15% on the resistant varieties. Thus, the varie-

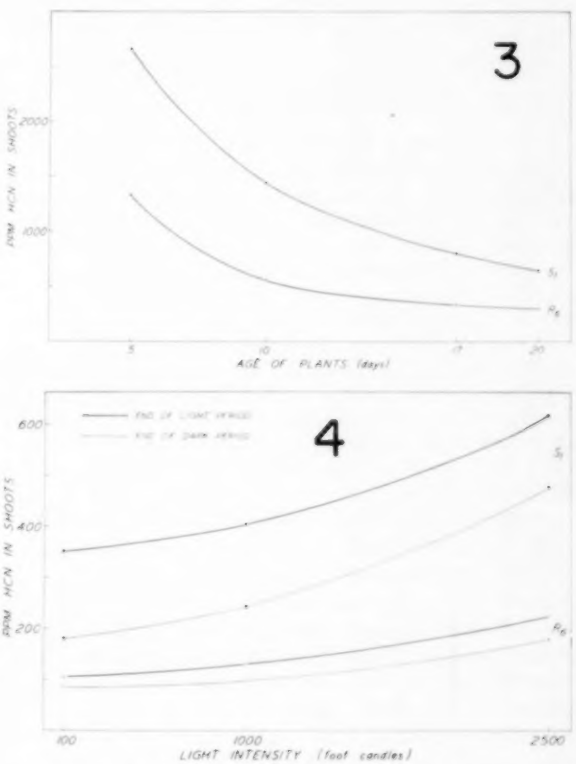

Fig. 3–4.—3) Variation in HCN content with age in 2 flax varieties (S_3 and R_2) grown at 20°C, 1200 ft-c, and 16-hour photoperiod. **4)** Effects of light intensities and light and dark periods on HCN content in 2 flax varieties (S_3 and R_2) grown at 20°C and 12-hour photoperiod.

ties listed as susceptible and resistant were respectively highly susceptible and highly resistant. Reference to Fig. 2 indicates that varieties resistant to clone 33-1 were not higher in HCN than susceptible varieties; in fact, the opposite is suggested. The present study indicates that high cyanide content of healthy flax varieties has no uniform correlation with resistance to either clone 294 or clone 33-1 of the pathogen.—Department of Agronomy, University of California, Davis.

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INFLUENCE OF SUBSTRATE ON SPORE SIZE OF ALTERNARIA MELONGENAE

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SUMMARY

The spore size of *Alternaria melongenae* varied significantly with source: from leafspot of eggplant, fruit scab of eggplant, or fruit rot of chili pepper. When isolates from the three natural sources were cultured on PDA and Richard's agar (RA), spore size was reduced markedly. Spore length was reduced in all cases. Spore breadth of the eggplant leaf isolate was reduced on PDA and RA; that of the eggplant fruit isolate was not significantly changed; that of the chili fruit isolate was reduced on RA but not on PDA. Beak length of the eggplant leaf isolate was markedly reduced on PDA and RA; that of the eggplant fruit and chili fruit isolates was reduced significantly on RA but not on PDA.

A report of leafspot and fruit scab of eggplant (*Solanum melongena* L.) and fruit rot of chili (red) pepper (*Capsicum annuum* L.) caused by a new species of *Alternaria*—*A. melongenae* Rang. & Samb.—is being published elsewhere (8). Later studies have showed considerable variation in the size of the fungal spores on the three natural substrata, eggplant leaf, eggplant fruit, and chili fruit (9). It is well known that spore size is influenced by nutritional content of the substratum, among other factors. Dosdall (3) and Christensen (2) showed that the spore size of *Helminthosporium sativum* P.K. & B. varied in different culture media. Sueda (10) reported on the influence of different nutrient media on the spore size of *Piricularia eryzae* Cav. Kulkarni and Patel (6) reported that the substratum considerably influenced the spore length and breadth of *Piricularia setariae* Nishikado.

Some species of *Alternaria* seem greatly influenced by nutritional factors; others do not. As early as in 1917 Elliott (4) found great variations in the spore size of 8 *Alternaria* species grown on several nutrient agar and natural host media; variation was more distinct in *A. iridicola* (Ell. & Ev.) Ell. and *A. sonchi* Davis. Tisdale and Wadkins (11), however, found the spore measurements of *A. longipes* (Ell. & Ev.) Tisdale and Wadks. to be essentially alike on 8 different media. Bolle (1) found considerable difference in the size of *A. brassicae* (Berk.) Sacc. and *A. solani* (Ell. & Mart.) Jones & Grout from different natural sources. Neergard (7) found that different media caused spore size to vary markedly in *A. brassicae*, *A. dianthi* Stevens & Hall, and *A. porri* (Ell.) Neerg., but not much in *A. anagallidis* Raabe, *A. matthiolae* Neerg., *A. senicionis* Neerg., and *A. zinniae* Pape.

Material and methods.—For study of the influence of substratum on spore size, 15-ml portions of PDA or Richard's agar (RA) were poured in 10-cm Petri dishes. Culture discs of the fungal isolates, cut with a 4-mm cork borer from rapidly growing regions of a 10-day culture in PDA, were inoculated in the center of the dishes and incubated 15 days at room temperature (23–25°C). Measurements were made of 100

spores selected at random from each dish. The data were statistically analyzed for significance of variation in length, breadth, and beak length of the spores, applying the Fisher's t test of significance (5).

Results. — The spores of eggplant leaf isolate measured 31.5–87.5 μ in length and 10.5–14.0 μ in breadth, and the beak length 10.5–52.5 μ. The respective modes were 44.1, 10.5, and 17.2 μ. The spores of eggplant fruit isolate measured 31.5–73.5 μ in length and 10.5–21.0 μ in breadth, and the beak length 3.5–31.5 μ. The respective modes were 54.1, 17.5, and 12.2 μ. The spores of chili fruit isolate measured 31.5–80.5 μ in length and 10.5–24.5 μ in breadth, and the beak length 7.0-45.5 μ. The respective modes were 44.1, 17.2, and 11.2 μ.

Statistical analysis of the above data showed no significant variation in spore length with substratum. Spore breadths, however, differed significantly with source, being broadest for spores from eggplant fruits and smallest for spores from eggplant leaf. Beak length was significantly longer for eggplant leaf isolate than for eggplant and chili fruit isolates, which were not significantly different.

Lengths, breadths, and beak lengths of spores resulting on PDA and RA and in nature were compared. Table 1 presents the results, statistically analyzed.

In general spore size was significantly less in pure culture than on natural hosts. Spore lengths of all three isolates were significantly reduced when cultured on RA and PDA. Spore breadth was significantly reduced in the isolate from eggplant leaf, but not in the isolate from eggplant fruit. Spore breadth

Table 1.—Measurements of 100-spore samples of *Alternaria melongenae* on hosts and 2 substrata.

Fungus source and substrate	Range (μ)	Mode (μ)	Mean (μ)	Standard deviation	Coefficient variability	t value at 5% when compared with host lesion	Difference*
Spore length							
Eggplant leaves							
On host	31.5–87.5	44.1	51.34	2.97	5.8		
On PDA	21.0–52.5	25.9	32.51	10.15	31.2	4.98	s
On RA	17.5–52.5	25.86	27.23	7.10	26.0	8.72	s
Eggplant fruits							
On host	31.5–73.5	54.1	49.17	9.24	18.7		
On PDA	21.0–49.0	33.6	33.56	6.3	18.7	4.42	s
On RA	24.5–56.0	31.15	34.47	6.65	19.2	4.03	s
Chili fruits							
On host	31.5–80.5	44.1	47.63	10.85	22.7		
On PDA	21.0–63.0	30.1	33.84	8.75	25.08	3.03	s
On RA	24.5–52.5	33.25	31.31	7.21	20.4	2.98	s
Spore breadth							
Eggplant leaves							
On host	10.5–14.0	10.5	10.74	0.56	5.2		
On PDA	3.5–14.0	10.5	9.20	1.96	21.2	2.38	s
On RA	7.0–14.0	10.5	8.92	1.82	20.3	2.73	s
Eggplant fruits							
On host	10.5–21.0	17.5	15.92	2.27	14.28		
On PDA	7.0–14.0	10.5	9.90	1.54	15.50	0.82	ns
On RA	7.0–17.5	10.85	11.58	10.25	88.50	1.30	ns
Chili fruits							
On host	10.5–24.5	17.15	15.43	3.57	23.12		
On PDA	7.0–14.0	10.15	9.31	2.03	21.8	1.72	ns
On RA	7.0–17.5	11.20	11.58	1.78	15.1	3.05	s
Beak length							
Eggplant leaves							
On host	10.5–52.5	17.22	23.38	9.13	39.9		
On PDA	3.5–21.0	10.71	8.82	4.62	52.3	4.00	s
On RA	3.5–28.0	8.85	7.28	4.65	63.2	4.40	s
Eggplant fruits							
On host	3.5–31.5	12.8	14.87	6.3	42.3		
On PDA	3.5–24.5	9.73	10.43	4.9	46.9	1.78	ns
On RA	3.5–21.0	8.75	9.24	4.55	49.2	2.30	s
Chili fruits							
On host	7.0–45.5	11.2	16.69	7.84	46.9		
On PDA	3.5–28.0	11.2	12.39	6.30	50.8	1.35	ns
On RA	3.5–17.5	8.75	9.90	5.81	58.6	2.36	s

* s, significant; ns, not significant.

of the isolate from chili fruit was significantly reduced by RA but not by PDA. Beak length of the eggplant leaf isolate was significantly reduced on both PDA and RA; beak length of eggplant fruit and chili fruit isolates were significantly reduced on RA but not on PDA.

Discussion. — The considerable influence of substratum on spore size, as also reported elsewhere (1, 2, 4, 5, 8), might be caused by more nearly complete nutrients in the natural hosts than the agar culture media. PDA and RA media seem to cause reductions in spore length of *A. melongenae* but not the breadth. Beak length of the spores was reduced on RA and not on PDA. Though the reasons for such reductions in spore and beak lengths are not known, it is probable that the components of the two agar culture media were at least partly responsible for the reductions. In RA, a synthetic medium, both spore breadth and beak length were reduced in all three isolates; in PDA, a complex medium, reactions of the three isolates were not uniform. Perhaps the various major and minor elements contained in the two media play a role in influencing spore size. It should also be noted that in no case was there a significant increase in spore size on the agar culture media. Though spore size is an important criterion for the speciation of fungi, spore size proved variable under nutritional influence. Care should therefore be exercised when this criterion is used for classifying fungi, especially the genus *Alternaria*.

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CONTROL OF CERCOSPORA LEAF SPOT OF BANANAS WITH APPLICATIONS OF OIL SPRAYS BASED ON THE DISEASE CYCLE

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SUMMARY

A method of surveying the intensity of Cercospora leaf spot of bananas, which permits the effective scheduling of oil spray applications for disease control, is described. The successive symptoms of the disease are classified as yellow streaks, brown streaks, and spots to provide an index as to when oil need be applied. Texaco 522 oil applied to yellow streaks halted further development into spots. The effectiveness of the oil lasted no more than 24 hours under field conditions. Oil did not prevent yellow streaks from appearing, nor did it effectively stop development of brown streaks into spots. This method has been successfully extended to control of the disease on 30,000 acres of bananas.

The displacement of Bordeaux mixture by petroleum oil for the control of Cercospora leaf spot of bananas introduced new questions about fungicidal action (4). Since the behavior of oil spray is different from that of Bordeaux mixture, it was proposed that scheduling of oil applications might also differ from that of Bordeaux mixture, which is applied at set intervals based on empirical observations made many years ago.

The results of Calpouzos et al (3) and Brun (1) were contradictory regarding the effect of oil on conidial formation and germination. Calpouzos postulated that oil inhibited the fungus at some stage inside the leaf after stomatal penetration had occurred and before symptoms had appeared. He also found that oil did not inhibit conidial germination or stomatal penetration. Brun concluded that oil was most effective in control when applied to "early brown streaks" even though he found that oil inhibited earlier symptoms. Brun also stated that oil inhibited conidial germination and sporulation.

Further studies were made to determine at what stage of the disease cycle oil exerted the most pronounced effects. Preliminary to these studies, it was necessary to obtain more data on development of the disease in the field.

Development of Cercospora leaf spot.—In the literature, the terms "flecks," "streaks," "spots," and "lesions" have been used with different connotations with reference to Cercospora leaf spot symptoms. Therefore various leaf symptoms were arbitrarily categorized as a basis for standardization of terminology and to facilitate identification of specific stages.

The categories are: 1) yellow streaks; 2) brown streaks; and 3) spots. Photographs of the various symptoms are presented in Fig. 1.

Studies were made on the disease cycle in the field. After conidial germination and penetration of the leaf, 7–49 days elapsed until the first macroscopic symptoms appeared, consisting of very early yellow streaks (Fig. 1-A) that were barely visible. Within 3–7 days, streak color changed from very light yellow to more intense yellow; the shape became more sharply defined, and the streaks enlarged as they entered the early yellow streak stage (Fig. 1-C). The color then changed from yellow to the tan (Fig. 1-D) of the early brown streak stage (Fig. 1-E); the streaks were completely tan 1–3 days after the transition began. Within the next 3–10 days, the tan color changed to dark brown, a thin black margin enclosed the streak, and mycelia were exposed from epidermal breaks, denoting advanced brown streaks (Fig. 1-F,G). A black border formed around the advanced brown streak, indicating the development of the early spot stage (Fig. 1-H,I). The transition through advanced spots (Fig. 1-J,K) to mature spots (Fig. 1-L) involved the formation of a gray center within the black border, sporulation, gradual increase in width of borders, and finally a desiccated white center. Calpouzos (2) reported that the period between first symptoms to the old spot stage varied between 10 and 60 days.

Effects of oil on disease development.—Observations were made to determine the effect of Texaco 522 oil applied at various disease stages. Texaco 522 oil is a paraffin-base lubricating oil having a viscosity of 99.3 SUS at 100°F, an unsulfonated residue of 85%, a neutralization number of 0.02, a flash point (open cup) of 370°F, and a density ($d^{20}4$) of 0.8785.

An experiment was initiated using 18 alternate plots (36 × 60 ft) of oil-sprayed and unsprayed banana plants. The total number of yellow streaks, brown streaks, and spots was counted on each leaf of 9 plants selected randomly from each treatment. Oil was applied at 2 gal./acre 0, 2, and 8 weeks after the original counts were recorded. After 10 weeks, the number of yellow streaks, brown streaks, and spots was again recorded. The final total count of streaks and spots combined was 4818 for the control and 4741 for the oil-sprayed plots. In the oil-sprayed plots, there were 3354 yellow streaks and 1397 brown streaks and spots. In the control plots, there were 877 yellow streaks and 3941 brown streaks and spots. Thus, yellow streaks on oil-sprayed leaves greatly outnumbered brown streaks and spots. On unsprayed leaves, most of the yellow streaks had developed into brown streaks and spots.

Most of the yellow streaks on oil-sprayed plants had a reddish tint. These proved to be yellow streaks that had been arrested in development. Brun (1) had also found streaks of reddish-chocolate color with arrested development. None of these reddish streaks were found on unsprayed leaves.

In another preliminary experiment in an unsprayed area, Texaco 522 oil was applied to single leaves with a DeVilbiss atomizer until the area to be sprayed was very lightly covered. Yellow streaks appeared within 1–30 days and developed into spots on leaves that had not had any symptoms of Cercospora leaf spot disease before the oil was applied. Development of yellow streaks into brown streaks was arrested when yellow streaks were present at the time oil was applied; however, new yellow streaks appeared within 24–48 hours of oil application, indicating that the effect of oil lasted no more than 24 hours. The efficiency of control was about 100% when oil was sprayed on yellow streaks.

A mature banana plant that has not yet borne fruit usually has 10–12 leaves. A new leaf develops every 7–14 days; within the same period the petiole of the oldest leaf loses its turgidity, the feaf folds, becomes non-functional, and ultimately dries or is cut from the plant. After the flower bud emerges, no new leaves form. A majority of the leaves already present must remain functional until the fruit is harvested (for about 90 days) or fruit weight is adversely affected. Cercospora leaf spot decreases the functional area of the leaf. Simmonds (5) found that leaf susceptibility to *Cercospora musae* varied directly with age of leaves; the younger leaves were more susceptible. In the present studies, the unfurled center leaf and the first and second open leaves were the most susceptible. Under farm conditions an average of 14–49 days from time of fungus invasion was necessary for most yellow streaks to develop. Based on these preliminary investigations, the total number of yellow streaks was recorded only for the second-, third-, or fourth-youngest open leaf, since these were normally the leaves with the highest frequency of yellow streaks.

When oil was applied before symptoms appeared, it failed to control the disease. When symptoms were in the border stage between yellow and very early brown streaks (Fig. 1-D) at the time oil was applied, about 50% of these streaks developed into spots and the remaining streaks were greatly retarded in their development. When symptoms were in the early brown streak stage (Fig. 1-E) at the time of oil application, only 20% of these streaks were controlled, although the rate of development of all of the remaining brown streaks was retarded. The development of advanced brown streaks into spots was slightly retarded but was in no case arrested. The development of viable and virulent conidia was not inhibited by oil applied to leaves having spots, or to leaves having brown streaks that subsequently developed into spots. When an excessive quantity of oil was applied to leaves with no symptoms or with yellow streaks, the effectiveness of the oil lasted a minimum of 12 days. Yellow streaks that appeared after oil was applied were arrested.

Farm-scale testing of control using the yellow-streak-count system.—In some oil-sprayed areas, Cercospora leaf spot was not efficiently controlled when oil was arbitrarily sprayed as often as every 7–10 days and some leaves had a minimum of 5–10 applications of oil. Therefore, an experiment was initiated to test the effectiveness of oil application under farm

conditions when applied according to development of yellow streaks. Appreciation is expressed to G. E. Donaldson, Spray Superintendent, Honduras Division, for his assistance in field experiments. The experimental design consisted of alternate plots in which were compared application of oil based on presence of yellow streaks and application of oil based on arbitrary spray cycles. Plots of Gros Michel bananas were 112 × 500 ft, excluding marginal areas or border rows. The experimental area consisted of 100 acres containing 12 replicates per treatment.

By cutting leaves from plants in border rows around the replicates and counting yellow streaks, the leaf with the greatest number of yellow streaks was determined. Subsequently, 2 leaves of similar position were cut from each replicate and all of the yellow streaks per leaf were recorded. In general, counts were made once or twice weekly on older plants that had not yet borne fruit. Further observations were made on the presence or absence of brown streaks and on the stage of deveolpment of the yellow streaks, i.e., whether very early (Fig. 1-A), early (Fig. 1-B), or advanced (Fig. 1-C). Arrested streaks that had an orange-red or violet appearance were not recorded.

The following criteria from sampling data were used as the basis for timing of oil application: a) spray when 50% of first or second open leaves have minimum of 50 advanced yellow streaks; b) spray when 50% of third- or fourth-youngest open leaves have minimum of 100 advanced yellow streaks.

Based on the above criteria, in the experimental plots, Texaco 522 oil was applied by helicopter at the rate of 0.8–1.2 gal. per acre per application.

These plots were not sprayed from March 19 through July 8. The control plots received 4 applications of oil during this period. Disease remained under control in both the experimental and the control plots.

Additional observations during this experiment were that yellow streaks continued to appear on individual leaves for an average of 4 days, after which time the maximum number of yellow streaks was found. This was followed by a period of 3 days when no new streaks appeared. The minimum period between peak quantities of yellow streaks was 7 days. It was essential to apply oil when the number of streaks was at a maximum, since the effectiveness of the oil lasted no longer than 24 hours. None of the calculated farm cycles would have been applied at the time of the maximum yellow streaks. In the experimental plots, oil was applied only when the number of streaks reached an average of 50 or 100 per leaf. Thus, some disease was continually present in these plots, but at no time was it out of control.

Discussion.—Based on results of field-scale tests, 30,000 acres of bananas in Honduras were placed under the new system of counting yellow streaks for timing of oil applications. Yellow streaks averaged 0–2000 per leaf in various locations on the second-, third-, or fourth-youngest leaves. When the number of yellow streaks was high, the volume of oil was increased from 0.8 gal./A. to 1.0–1.5 gal./A. in order to maintain good control, since it was found that low volumes did not give effective leaf coverage when streak-counts were high and streaks were not affected by oil when they were not covered. During the rainy season, it was impossible to spray exactly when desired in every case and it was therefore necessary to spray one or two days late in relation to streak stages. Subsequently, there was a reduction in the quantity of streaks arrested; however, a large number of streaks were retarded in development and changed into spots only on the lowest leaves. Because of the lower quantity of streaks arrested, the total amount of disease increased. However, control has not been lost in any of the areas under the yellow-streak-count system when oil coverage has been adequate.—Department of Plant Pathology, Vining C. Dunlap Laboratories, Tela Railroad Company (subsidiary of United Fruit Co.), La Lima, Honduras, C. A.

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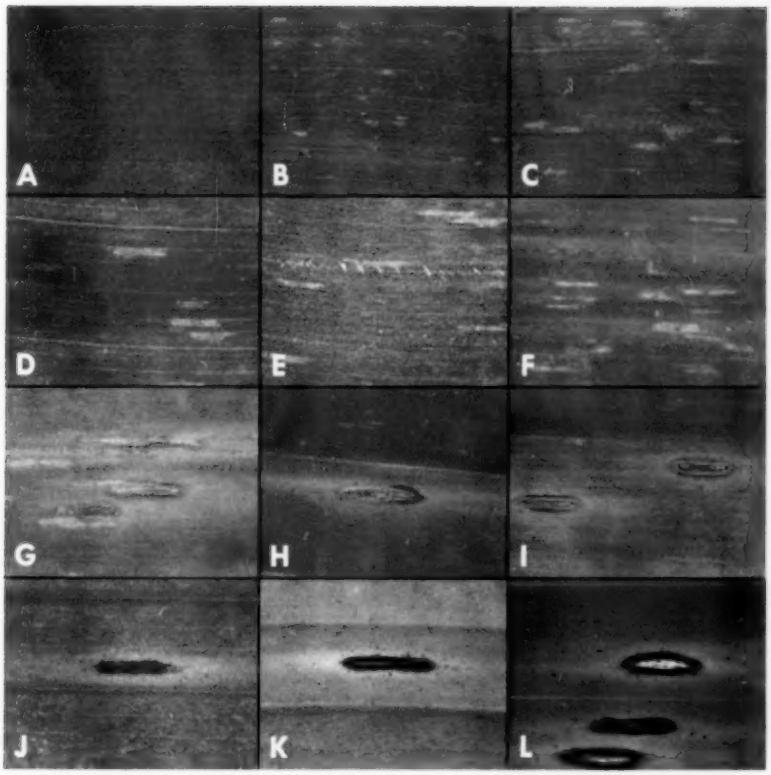
Fig. 1.—Stages of Cercospora leaf spot on leaves of mature Gros Michel bananas: **A)** very early yellow streak; **B)** early yellow streak; **C)** advanced yellow streak; **D)** borderline stage between advanced yellow streaks and early brown streaks; **E)** early brown streak; **F-G)** advanced brown streak; **H-I)** early spots; **J-K)** advanced spots; **L)** mature spots. (1.8×)


A
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INITIATION OF STRAWBERRY FRUIT ROT CAUSED BY BOTRYTIS CINEREA

R. L. Powelson

Technical Paper No. 1280, Oregon Agricultural Experiment Station. Part of a Ph.D. thesis, Oregon State College. The investigation was supported in part by a grant from the Birds Eye Division of the General Foods Corporation. Accepted for publication February 2, 1960.

SUMMARY

Botrytis rot, the most important fruit rot of strawberries in Pacific coast fields, usually originates at the stem end of the fruit. Isolations and histological studies indicate that *Botrytis* mycelium is present in senescent petals, stamens, and calyces of marketable fruit. Latent infection, confined principally to the stem end, was determined by isolation, incubation, and histological study of marketable and apparently disease-free fruit. Strawberries grown in the greenhouse under conditions favoring severe disease development were attacked less frequently by *Botrytis* if petals, stamens, and calyces were removed after fertilization. Three preharvest applications of captan significantly reduced rot incidence in the field, and also the amount of latent infection of marketable fruit. The difference in rot incidence between treated and untreated plots remained nearly constant throughout the wet 1958 harvest season. The failure of this difference to decrease with the increasing number of rotting fruits and airborne spores toward the end of the harvest period indicates a minor importance for direct infection of ripe fruit by airborne spores.

Introduction. — Botrytis rot, caused by *Botrytis cinerea* Pers., is the most important fruit rot of strawberries in Pacific coast fields. Losses are most serious when rainfall is excessive during the harvest period. Powelson (10) found that *B. cinerea* caused over 90% of the fruit rot in fields surveyed in British Columbia, Washington, Oregon, and California in 1956, 1957, and 1958. Minor amounts were caused by *Rhizopus stolonifer* (Ehr., ex Fr.) Lind., *Dendrophoma obscurans* (E. & E.) Ander., *Gnomonia fructicola* Arn., *Rhizoctonia* spp., and a *Melanconium* sp.

The disease, while regarded as a fruit rot, often causes a blossom blasting and rotting of green fruit during extended rainy periods or high humidity (1, 8, 12, 13). Field observations indicate that much of the fruit rot results from infection spreading from petals and sepals to the fruit (4, 5, 7, 14, 15).

Experimental results.—*Infection of floral organs.* —A survey of 5 strawberry fields in the Willamette Valley showed 71–87% of the rotting fruits infected at the stem end. In many cases only one side of the stem end was involved, and the rot appeared to have originated from infected stamens or sepals. To establish whether these floral organs were pathways for fruit infection, isolations were made from symptomless green and ripe fruit and from attached necrotic and non-necrotic floral organs. Isolations were made by stemming surface-sterilized fruit and transferring a small piece of exposed tissue to Petri plates containing strep-PDA (potato-dextrose agar containing 50 ppm streptomycin-nitrate). Whole petals, stamens, and sepals were also surface-sterilized and transferred to Petri plates contraining strep-PDA. Necrotic petals, stamens, and sepals, as well as symptomless ripe fruit, were frequently infected by *B. cinerea* (Table 1).

Table 1.—Frequency of isolation of *Botrytis cinerea* from symptomless green and ripe fruit, and from attached necrotic and non-necrotic floral organs.

	% fruits		% floral parts	
Floral organ	Green	Ripe	Necrotic	Non-necrotic
Petal			80.0	4.4
Stamen			43.3	3.3
Sepal			23.3	8.9
Receptacle	6.7	53.7		

To determine whether this latent infection of symptomless fruit was associated with areas other than the stem end, fruits were surface-sterilized and cores perpendicular to the fruit axis were taken with a sterile 5-mm cork borer, from the stem end, middle, and tip end. A thin epidermal slice was removed from each end of the fruit core and the core was plated on strep-PDA. Isolations made from fruit samples harvested early, middle, and late in the season show that 74% of the latent infections were confined to stem end tissues (Table 2).

Further evidence for the hypothesis that floral parts are infection loci was obtained by greenhouse experiments. From Marshall strawberry plants paired berries in the same fruit cluster were selected, and the petals, stamens, and sepals were removed from one berry of each pair. The fruits were selected 5–10 days after fertilization, and those later showing signs of malformation were discarded. Previous observations indicated inconsistent rot development in malformed fruits. Plants with the above paired fruits were then grown under various combinations of environmental conditions in an attempt to simulate weather conditions that might occur during the blossom and harvest periods. The plants were divided into four groups. Two groups were placed inside a moisture chamber, where they received intermittent mist from overhead spray jets, and the other two groups remained outside the chamber under dry greenhouse conditions. After 2 weeks, plants to receive a "wet-bloom–dry-harvest" treatment were removed from the moisture chamber and exchanged with the plants to receive a "dry-bloom–wet-harvest" treatment. A high density of air-borne *Botrytis* spores

Table 2.—Distribution of latent infection by *Botrytis cinerea* in marketable strawberries.

	No. isolations in 30 attempts		
Date sample taken	Stem-end	Middle	Tip
May 26, 1958	16	4	0
June 4, 1958	17	4	0
June 30, 1958	21	9	2

Table 3.—The effect of removing petals, stamens, and sepals on the incidence of fruit rot under various combinations of wet and dry environmental conditions in the greenhouse.

Environmental sequence[a]	Floral Parts	Initial no. of fruits	Symptomless at maturity
Dry–dry	Attached	20	16
	Removed	20	19
Dry–wet	Attached	24	4
	Removed	24	12
Wet–dry	Attached	23	1
	Removed	23	13
Wet–wet	Attached	19	1
	Removed	19	7

[a] An attempt was made to simulate various combinations of weather that might occur during the blossom and harvest periods.

was artificially maintained in the experimental areas by placing rolled oats inoculated with *B. cinerea* on a platform 12 in. from each plant. Table 3 shows the number of fruits that ripened without showing symptoms of rot. The total number of fruits with no symptoms of rot at maturity under the 4 sets of environmental conditions was 51 if petals, stamens, and sepals had been removed, and 22 if not removed; respective numbers under continuous and partially wet environments were 37 and 6 (Fig. 1).

Histological evidence of infection.—Histological studies were made to determine the site and method of infection. Naturally infected Marshall strawberries with symptoms of petal, stamen, or sepal infection, but no apparent stem end rot, were examined. Tissues for sectioning were immersed in Rawlins (11) No. 2 formalin–acetic-alcohol killing and fixing solution, and infiltrated and embedded in paraffin according to the tertiary butyl alcohol method of Johansen (3). Sections 14 μ thick were cut and stained after a hematoxylin-safranin schedule. Direct mounts of fresh material were made by placing pieces of tissue for 1–2 minutes in heated (fuming) lactophenol containing 1% cotton blue or acid fuchsin and then mounting in clear lactophenol. Pieces of tissue adjacent to those examined histologically were plated on strep-PDA for positive determination of *B. cinerea*.

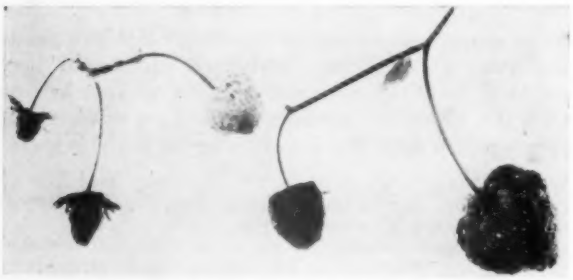

Fig. 1.—Effect of petal, stamen, and calyx removal on susceptibility to rot under wet conditions. The fruit on right of each cluster had perianth and stamens removed shortly after fertilization, with calyx remaining attached to the others.

Hyphae were present in necrotic petals, stamens, and sepals (Fig. 2-A,B,C). Invasion of the stem end of the receptacle (Fig. 2-D) originated in infected stamens and sepals—in most cases, primarily from infected stamens. Invasion of the calyx tissue below the point of stamen attachment (Fig. 2-E) occurs within the 2–3 cell layers inside the epidermis, as evidenced by hyphae and separation of cells in this area (Fig. 2-C). Latent infection of the stem end of the receptacle appeared to be limited to a radial spread of hyphae in parenchyma tissue between the epidermis and the major vascular network of the fruit. Hyphae were more numerous in tissues adjacent to the vascular bundles (Fig. 2-F).

There was no indication that primary infection originates from direct infection of the fruit through the epidermis of the stem end, or from contact of infected stamens and sepals with the fruit surface. The fruit may be infected directly from petals trapped beneath the calyx when conditions are conducive to severe petal blight. An abscission layer usually causes the petals to fall before the fruit is invaded.

Effect of fungicides on latent infection.—Several workers have controlled strawberry fruit rot with preharvest applications of fungicides (2, 8, 14). Three preharvest applications of captan spray significantly reduced the incidence of fruit rot in 1958 tests of fungicides on Marshall strawberries for rot control at Hillsboro, Oregon. Incidence of fruit rot for each picking is graphed in Fig. 3. Conditions favored fruit rot during the harvest period (May 25 to June 13), during which 3.22 inches of rain fell on 12 days, with no more than one day at a time without precipitation. The difference in rot between treated and untreated plots remained about the same throughout the harvest period (Fig. 3).

To determine the effectiveness of field application of fungicides in preventing infection, a cooperative spraying and dusting program was conducted in 1958 with the help of Mr. W. B. Neuburg (Research Horticulturist, Western Branch Laboratory, Birds Eye Division, General Foods Corporation, Hillsboro, Oregon). Data were taken on the incidence of field rot and

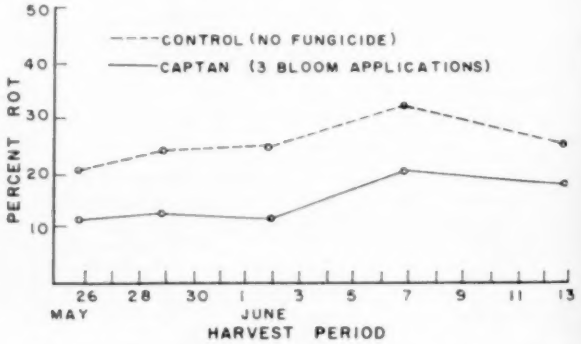

Fig. 3.—The effect of 3 preharvest applications (April 22, May 6, and 21) of captan (3 lb of 80% wettable powder per 100 gal. per acre) on the incidence of strawberry fruit rot at each picking during the harvest period.

latent infection of marketable fruit. Latent infection was determined by the following procedure: 1) samples of 100 marketable fruits with calyx attached were taken at the time of the second picking (June 4, 1958)

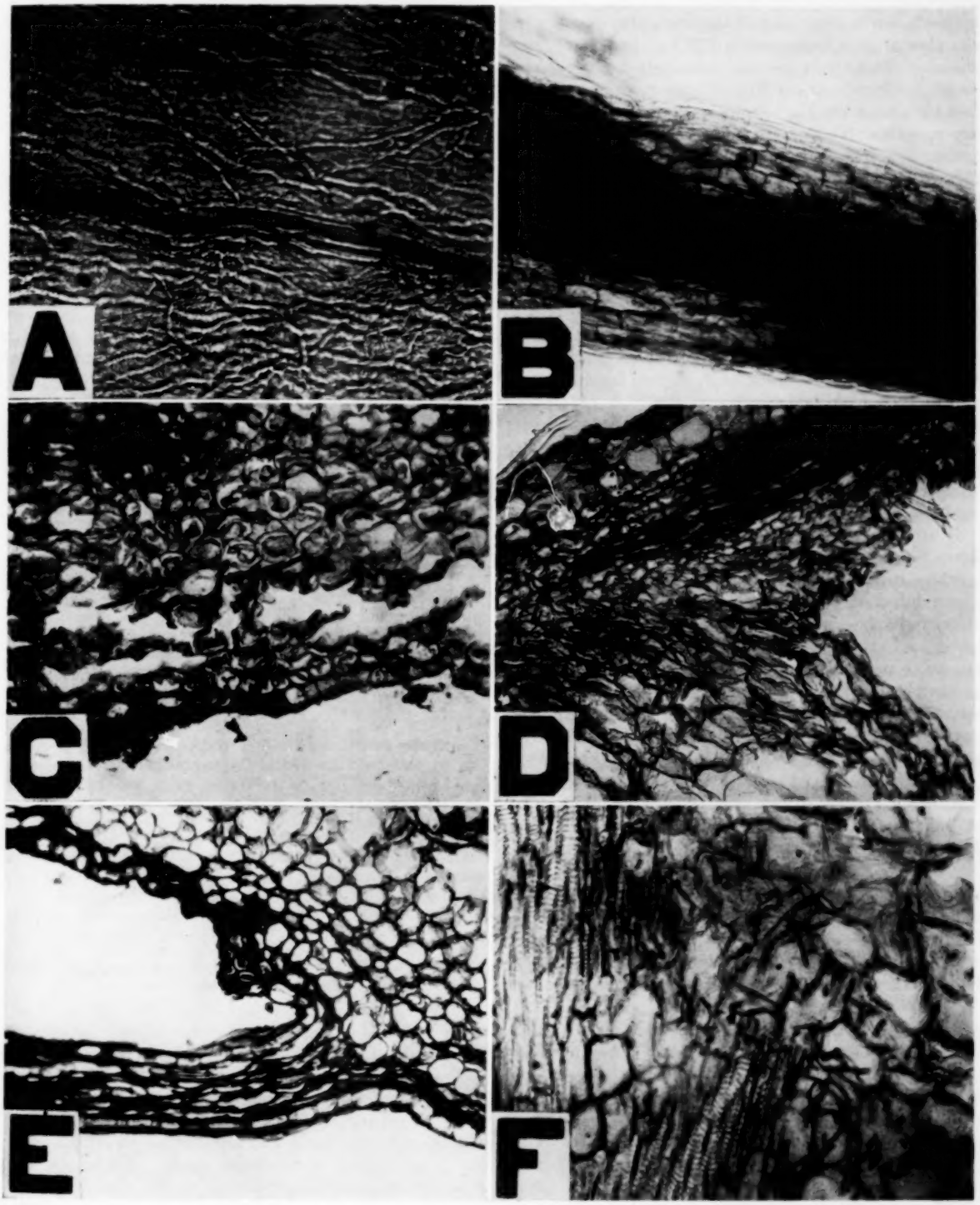

Fig. 2.—Strawberry floral organs infected by *Botrytis cinerea*. **A)** Hyphae in petal (×100). **B)** Hyphae in filament (×100). **C)** Sepal showing hyphae in the subepidermal layers below the lower epidermis and separation of cells (×200). **D)** Hyphae in stem end of receptacle in the region of calyx attachment (×100). **E)** Necrotic stamen and sepal at point of filament attachment (×100). **F)** Stem end of receptacle showing extensive invasion of hyphae adjacent to vascular tissues (×200).

from each replication of treated and untreated plots; 2) fruits were washed in water containing a detergent; 3) rinsed in tap water; 4) soaked about 1 minute in 20% Clorox (sodium hypochlorite; 5.25%); 5) rinsed in fresh tap water; and 6) incubated in a moisture chamber at room temperature (72°F). In the moisture chamber each fruit rested separately on absorbent paper saturated with a 20% Clorox solution to maintain high humidity and minimize the chances of secondary infection. The number of fruits showing signs of fungus infection was recorded after 4 days. Marketable Marshall strawberries from plots that received 3 preharvest applications of captan dust (35 lb of 7½% dust per acre on April 23, May 6, and May 26) developed 27% fruit rot, compared with 85% for fruits from plots that received no fungicide. In the field, these same plots respectively developed 10.0 and 29.5% rot.

Discussion.—Botrytis rot of strawberries is characterized in most cases by the origin of rot at the stem end. The frequent isolation of *B. cinerea* from the stem end of ripe marketable fruit and the high percentage of these fruits that rot under moist conditions indicate considerable latent infection. In most cases, necrotic stamens and sepals were associated with marketable fruit having latent infection, and these necrotic floral parts were frequently infected by *Botrytis*.

It is generally recognized that infection of strawberry floral organs by *B. cinerea* is common, but most workers consider that the major losses are caused by direct infection of the ripening fruit (1, 2, 7, 9, 14). *Botrytis* hyphae were observed in infected petals, stamens, and calyces, and also in the stem end receptacle tissue of marketable fruit. The receptacle appeared to have been infected from stamen and calyx infection, with invasion being internal through these tissues and not by contact of the infected floral parts with the fruit surface.

The infection of senescent floral parts by *B. cinerea* provides a source of mycelium that is capable of invading the tissues of the healthy strawberry fruit. Removal of petals, stamens, and calyces shortly after fertilization markedly reduced the incidence of fruit rot and demonstrated the importance of those parts as pathways by which *B. cinerea* gains entrance to the fruit.

That the fungicide protects against petal, stamen, and calyx infection seems most probable since the surface area of the small fruit that received the fungicide would have expanded many times between the time of application and fruit maturity. Residue analyses made by Martin and Pickard (6) show only 8 ppm of captan on marketable fruit harvested 20 days after the last of 3 preharvest applications of captan, 400 ppm in the calyx. This protection of the calyx by the fungicide would account for the comparatively low percentage of rotted fruit from plots receiving preharvest captan sprays, and adds further evidence that the calyx is the primary pathway of infection.—Department of Botany and Plant Pathology, Oregon State College, Corvallis.

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THE EPINASTY VIRUS REACTION OF KWANZAN AND SHIRO-FUGEN FLOWERING CHERRY

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SUMMARY

The virus causing an epinasty reaction of Kwanzan and Shiro-fugen flowering cherry is shown to be the green ring mottle virus and not ring spot virus. The green ring mottle and ring spot viruses were separated by field selection, mechanical transmission, and heat therapy. The two viruses in separate cultures gave distinct reactions on Shiro-fugen and Kwanzan flowering cherry.

When buds were taken from apparently normal stone fruit trees and budded into Shiro-fugen and Kwanzan flowering cherry (*Prunus serrulata*), a local necrotic reaction often occurred about the bud on Shiro-fugen, and buds from the same source placed on Kwanzan caused epinasty (2). Milbrath and Zeller (4) were the first to associate these reactions with the latent virus complex in stone fruits without reference to a specific virus. In an earlier paper (3) they described and illustrated a rough-bark disease of Kwanzan with leaf epinasty as one of the symptoms. More recent work with the same source tree has demonstrated that the virus causing epinasty in Kwanzan is present in the culture and that a specific strain of this virus may have caused rough-bark. Moore (J. Duaine Moore, University of Wisconsin, in personal correspondence in 1950–52) maintained that most of his cultures did not cause Kwanzan epinasty, but when they did they also caused green ring mottle (5) on Montmorency. He did not believe that the epinasty reaction was caused by necrotic ring spot virus, but Milbrath used the varying degree of severity of epinasty as an indication of mild to severe strains of the ring spot virus (2). Fridlund and Diener (1) concluded that the virus causing epinasty was distinct from the necrotic ring spot virus and was probably the cause of green ring mottle of Montmorency.

The data presented herein are additional evidence that the local necrotic symptom in Shiro-fugen is caused by ring spot virus but that the epinasty symptom in Shiro-fugen and Kwanzan is caused by green ring mottle virus.

Separation by field selection.—Thirty-two trees from several different areas and representing several species of *Prunus* were selected as virus sources. Bark buds from each of the 32 sources were placed in Lovell peach seedlings to facilitate virus recovery on herbaceous hosts. The mechanically transmitted virus associated with stone fruit ring spot was readily isolated from the 26 sources that gave a local necrotic reaction on Shiro-fugen. Many attempts have been made to recover a virus on cucumber and squash from the 6 trees with only epinasty reaction, but all have been negative.

No foliage symptoms were induced when buds from the 6 trees causing only epinasty in Shiro-fugen were inserted into Bing, Lambert, Black Republican, Napoleon sweet cherry, Early Muir peach, and Italian prune. No necrotic ring spot shock reaction or any other foliage symptoms were noticed in Montmorency the first year following fall inoculations, but all sources caused green ring mottle symptoms in subsequent years.

Separation through mechanical transmission.—A virus was recovered in squash by juice inoculations from 7 of the 12 source trees that originally gave both an epinasty reaction on Kwanzan (Fig. 1-A) and a local necrotic reaction on Shiro-fugen. Small Mahaleb seedlings were inoculated with juice from the squash, and, when the plants were large enough, buds were removed and placed onto Shiro-fugen. One or more plants for each source were infected with a virus that caused a local necrotic reaction on Shiro-fugen, but no epinasty symptoms were observed on inoculated Kwanzan trees even when held for the second growing season. Instead scattered Kwanzan leaves showed a mild spot mottle or fine zonate line patterns (Fig. 1-B). None of the leaves showed any distortion, necrosis, or epinasty. Apparently, the virus responsible for the epinasty reaction was not transmitted mechanically and only the ring spot virus was present.

Separation by heat treatment.—Two viruses were accidentally separated during an attempt to free Napoleon sweet cherry trees of virus by prolonged heat treatment of the living plants. Young trees were propagated by spring-grafting mazzard seedlings. The Napoleon scions were taken from a tree known to be infected with the virus or viruses that would cause local necrotic reaction on Shiro-fugen and systemic epinasty reaction on Kwanzan. By fall the plants were 12–18 in. tall. When the terminals had become dormant, they were placed in a heat chamber in about 98–100°F. Scions were removed at 4–5-day intervals, and 5 buds from each were placed in Mahaleb seedlings. The surviving buds were allowed to grow for one season and then removed and placed in Shiro-fugen for the local necrotic reaction. All trees grown from buds heated fewer than 13 days gave a local necrotic reaction on Shiro-fugen. Two trees developed from buds that had been heated 13 days were both negative on Shiro-fugen. Four buds produced trees after 19 days' treatment, and 2 of the trees gave the necrotic reaction on Shiro-fugen and 2 did not. The Shiro-fugen negative trees were then tested for the epinasty reaction on individual Shiro-fugen trees by a quick index method similar to that reported by Williams and Wagnon (6). All caused a leaf epinasty, but in no case did necrosis develop at the graft line. The ring spot virus responsible for the necrotic reaction on Shiro-fugen had apparently been destroyed during the heat treatment, leaving only the virus that caused the epinasty reaction.

G. Nyland (Regional Stone Fruit Virus Project W-22, Annual Report of California contributing proj-

ect, 1958) obtained similar separations of the ring spot virus and green ring mottle virus in heat treatment experiments.

Discussion and conclusions.—These studies demonstrate that varying degrees of epinasty in Kwanzan is not an indication of mild and severe strains of ring spot virus, as previously proposed (2). The epinasty virus is common in stone fruit trees in the western United States, often mixed with the ring spot virus. Interaction of these two viruses may be responsible for the range of severity of the epinasty virus, varying from a very mild epinasty on full-size leaves to a very severe dwarfing and curling of the leaves the season following inoculation and eventual death of the Kwanzan above the point of bud insertion.

These data support the contention that the epinasty

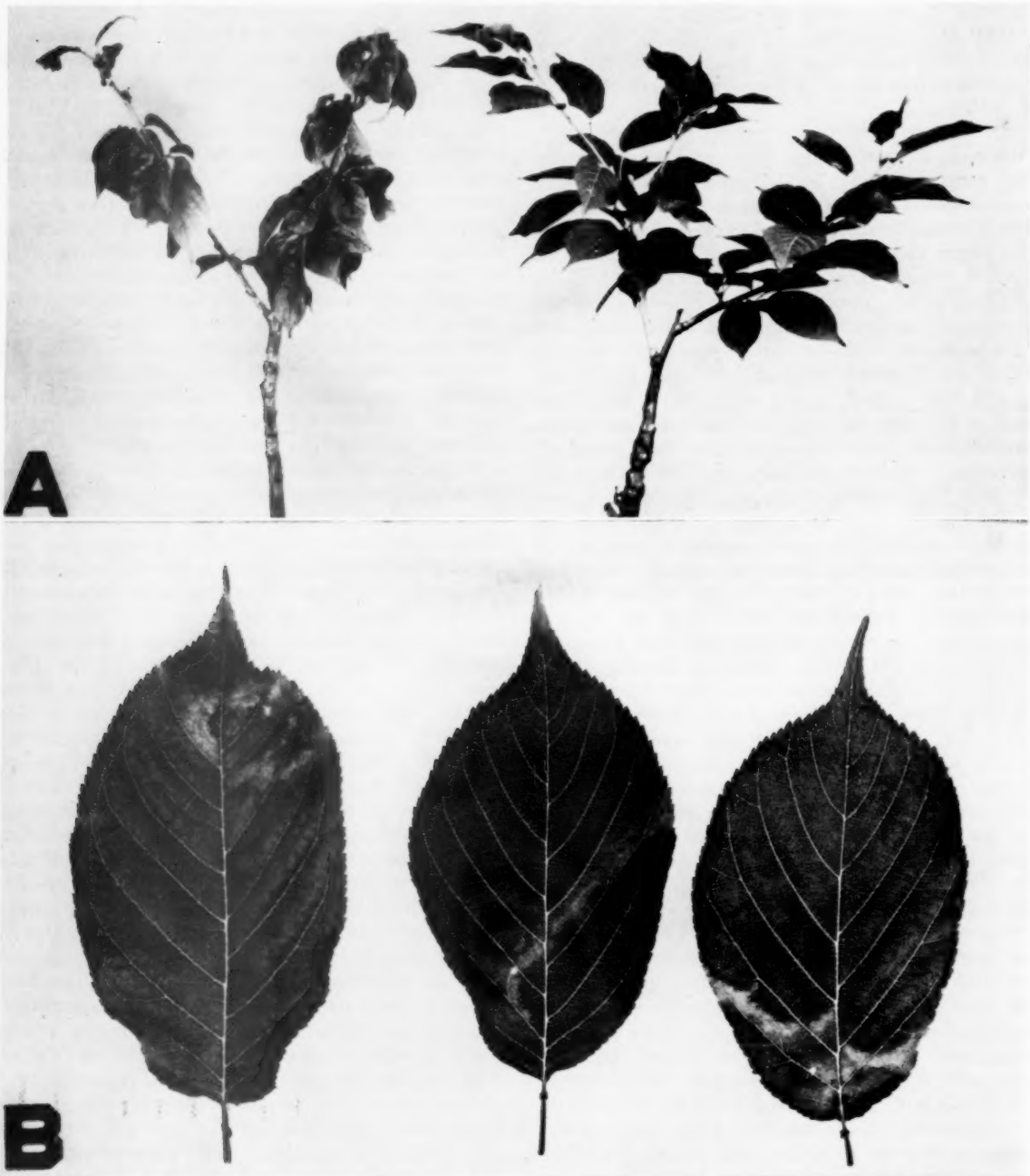

Fig. 1.—**A)** On left, epinasty symptom in Kwanzan plant after inoculation with buds from original source tree plant. Plant on right shows no epinasty after inoculation with a Mahaleb mechanically inoculated with a virus from Buttercup-squash. The squash had been infected with the ring spot virus from the same source tree used to inoculate tree on left. **B)** Leaves, from Kwanzan on right above, showing the mild leaf mottles and zonate lines.

reaction on Shiro-fugen and Kwanzan is caused by the green ring mottle virus. When Montmorency trees were inoculated with unheated buds of the Napoleon source tree used in the heat inactivation experiments, they developed necrotic ring spot shock symptoms the first year and green ring mottle symptoms in the following years. Heat inactivation eliminated ring spot virus from the buds, but epinasty showed that the green ring mottle virus was still present.

The epinasty reaction on either Shiro-fugen or Kwanzan is a more reliable index for green ring mottle virus than the green ring mottle symptoms, which develop slowly on Montmorency, and often more erratically. Kwanzan is the better host if the culture is contaminated with ring spot virus. If Shiro-fugen is used with such a culture, the ring spot virus will often kill the tree before epinasty can be expressed. When indexing for virus freedom, Shiro-fugen would be a better host since a failure to induce either necrosis or epinasty would indicate the test plant to be free of both viruses.—Department of Botany and Plant Pathology, Oregon State Colege, Corvallis.

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TOBACCO MOSAIC VIRUS IN NICOTIANA GLAUCA

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SUMMARY

Nicotiana glauca often invades areas in Southern California where the indigenous vegetation has been disturbed. Many bushes are infected with viruses, including a form of tobacco mosaic virus (TMV). TMV isolates from *N. glauca*, obtained by mass transfer or through single lesions on inoculated *N. glutinosa*, have consistently had host reactions of the U2-U5 type of TMV. They are similar to some TMV isolates from *N. glauca* growing wild in other regions of the northern hemisphere. As a host plant, *N. glauca* seems to encourage a form of TMV, a variant of which (U2) may exist as a minor component in the TMV complex occurring naturally in tobacco. The *N. glauca* form of TMV differs markedly in host reactions and biophysical properties from the dominant form in tobacco.

Characteristic isolates of tobacco mosaic virus (TMV) that cause severe symptoms in *Nicotiana tabacum* L. produce ephemeral or restricted symptoms or none in *Nicotiana glauca* Graham (3). Yet that species suffers a widespread disease with distinct symptoms, and forms of TMV differing from those dominant in tobacco have been isolated from diseased plants (10, 11). That such forms may be largely responsible for the disease is borne out by observations in southern California.

The incidence and seasonal variation in symptoms were watched in a population of infected *N. glauca* bushes, concentrated mainly beside a field along the edge of an indigenous hillside cover in the Ornamental Horticulture Area on the Los Angeles campus of the University of California (UCLA). Groups of *N. glauca* also became established within the hillside association, after it was seriously disturbed several times. The whole area was roughly triangular and of about two acres. Other, less permanent stands of *N. glauca* nearby and throughout southern California have been observed, and some sampled, during the last 10 years.

TMV isolates from N. glauca.—*The single-lesion isolate, U5.*—Inoculations from infected *N. glauca* bushes on the UCLA campus were made to tobacco, *N. glutinosa* L., and other host species. In 1950 the senior author obtained single-lesion isolates from very dilute inoculum, and one of them, causing a yellow mosaic in *N. glauca* (Fig. 1), was chosen as representative of the isolates causing the most striking and easily identified form of the disease. It was included by Siegel and Wildman (13) as one of 8 strains of TMV studied in detail, and was designated U5. It was characterized and compared with the 7 other strains, which included typical single-lesion isolates of severe (U1) and mild (U2) strains from tobacco. The U1 and U2 strains represent the major and minor components in Johnson's standard sample of TMV (14, 8). The *N. glauca* isolate, U5, is almost indistinguishable from U2 on the basis of biophysical properties (13), but can be distinguished by differences in symptom expression. On *N. glauca*, U2 is symptomless. Both U2 and U5 are clearly differentiated from U1 by their biophysical properties (13), and by the reactions of certain host plants, notably *N. sylvestris* Spegaz. and Comes and Turkish tobacco (11). U1 is systemic in such plants and produces occasional mutant yellow spots on mature infected leaves; U2 and U5 produce only necrotic local lesions in *N. sylvestris*, and no mutant yellow spots in systemically infected Turkish tobacco. A "mild dark-green" and a yellow isolate of

TMV from *N. glauca* (11) induced in these 2 host plants reactions like those from U2 and U5.

Comparison with a yellow mutant of U1 from N. glauca.—In McKinney's studies (11) a TMV isolate of the U5 type from *N. glauca* was compared in the greenhouse with a yellow mosaic of the U1 type, which was also isolated from *N. glauca*. The same sort of comparison was made with UCLA material. Inoculation of *N. glauca* with U1 and other similar TMV isolates from tobacco gave almost symptomless but systemically infected plants. They characteristically had occasional yellow or yellow-green mutant spots. An isolate from a yellow-green spot taken through *N. glutinosa* and tobacco gave in *N. glauca* mildly chlorotic local lesions and a systemic mottle that was somewhat less mild than the faint mottle of U1, and occasionally included definite yellow areas. The progress of symptoms during two years was watched in 16 plants held first in the greenhouse and later outside. Outside there was an erratic increase in severity to a restricted yellow mottle on occasional leaves. In spite of this, and although the mutant arose in the tissues of *N. glauca*, its concentration in young leaves was low and symptoms on vegetative shoots often lagged behind the growing point.

In *N. glauca* under the same conditions U5 gave an aucuba mottle without definite rugosity or distortion of the leaf outline (Fig. 1). The mottle appeared first on young leaves as light and dark green areas. The regular and distinctive symptoms of U5, developing on *N. glauca* without serious reduction of growth, indicated a form of virus well adapted to its host.

In Turkish tobacco the U1 mutant gave a strong yellow mottle; in later-formed leaves the mottle was light and dark green, and very little brighter than that of U1. The characteristic symptom of U5 was rugosity (Fig. 2); intensity of mottle was much less than from U1. As well as could be judged by local-lesion trials, both U5, with milder symptoms than from U1, and the mutant form of U1 from *N. glauca*, with brighter symptoms than from U1, produced concentrations of virus in tobacco less than those from U1 itself (Bald, unpublished). Turkish tobacco was a moderately good systemic host for U5 and the *N. glauca* yellow mutant, but it was a better host for U1; whereas *N. glauca* appeared better adapted to U5 than to U1 or the U1 yellow mutant (see also (11)).

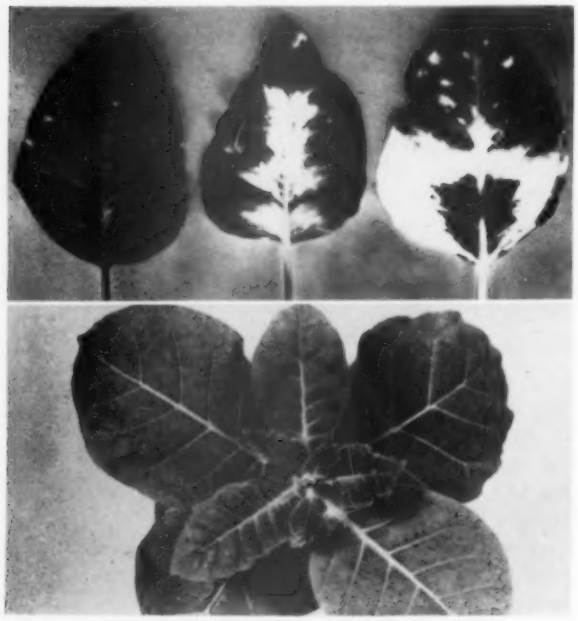

Fig. 1–2.—**1)** Three *Nicotiana glauca* leaves with symptoms of TMV strain U5 isolated from *N. glauca*. **2)** Symptoms of TMV strain U5 on Turkish tobacco.

Further inoculations from infected N. glauca bushes.—After 10 years' observation a final check was made to discover whether TMV isolates of the U5 type still appeared dominant in the TMV-infected *N. glauca* bushes on the campus. Isolates from 6 representative infected bushes were studied and found to be of the U5 type. They varied considerably in symptom expression, but all gave necrotic local lesions on *N. sylvestris*. The results were similar to those from earlier inoculations. There had been no major change in the form of TMV infecting the UCLA population of *N. glauca* during those 10 years.

Seasonal observations.—The *N. glauca* on the UCLA campus represent by far the most stable population of any size in which observations on infected *N. glauca* plants have been made. Further, it is the one in which yellow isolates of TMV have been most nearly dominant. Since 1948–49 the proportion of plants with bright yellow mottle has increased rather than decreased and symptomless plants are now few.

Each season when conditions were favorable, seedlings of *N. glauca* emerged beside the infected bushes, one of which had yielded U5. The seedlings were generally cut back, and regrew once or twice before they were weeded out. Many became infected with viruses. The symptoms on a large number of infected young plants were similar to those on the young plants inoculated with the single-lesion TMV isolate, U5. U5, by these uncontrolled tests, was shown, at least in symptom expression, to be characteristic of types of TMV in the stand of *N. glauca* from which it originally came.

Few of the *N. glauca* seedlings have survived, and a seasonal cycle of symptoms prevails in older bushes. On the new growth of spring and early summer, mottle occurs close to the growing point of vegetative shoots; on many plants light-green areas become more yellow as the leaves age. Small leaves on older flowering branches also have symptoms. The natural tendency to shed leaves from woody twigs during long, dry spells seems to be enhanced by infection, particularly by mixed infections, in which TMV may be combined with another virus; but the leaf fall is not enough to weaken the plants seriously. The U5-type TMV isolates appear well adapted to invasion of most shoots and leaves, although a symptomless young shoot is occasionally found on a long-infected plant. The tolerance of *N. glauca* to virus infection is sufficient to allow persistence of established infected plants for

years in partial competition with other shrubs; e.g., *Rhus laurina* Nutt. and elderberry.

Distribution.—The basis for diagnosing the U5 form of TMV in naturally occurring *N. glauca* bushes is: 1) U1 and similar isolates do not produce in *N. glauca* the same range of symptoms as do the isolates related to U5, and they can be discounted as the cause of an obvious mosaic disease of this species in southern California; and 2) sampling has given TMV isolates that consistently produce necrotic local lesions on *N. sylvestris*, whatever their symptoms on *N. glauca*, tobacco, and other species.

The yellow mottle produced in *N. glauca* by U5 is a standard symptom for distinguishing with reasonable probability between similar types of TMV and the ordinary severe tobacco strain and its derivatives. Isolates of the U5 type, causing light- and dark-green mottles, were also diagnosed correctly in a high proportion of examples tested. This may be illustrated by a sampling of *N. glauca* bushes from within an area of 2–3 square miles near Riverside, California. Fifteen bushes were selected as infected with TMV of various degrees of severity, and probably also with a second virus. Of 12 tested by inoculation, 10 were shown to contain TMV. Single-lesion isolates of TMV derived from 2 *N. glauca* plants were transferred through *N. glutinosa* to tobacco. Symptoms varied widely. Six of the single-lesion isolates, ranging in severity from the mildest to most severe, were inoculated to *N. sylvestris*, Turkish tobacco, tobacco with the NN factor, and *N. glutinosa*. All gave systemic symptoms on Turkish tobacco and only necrotic local lesions on the other 3 host plants. These 6 representative TMV isolates were of the U5 type.

From such diagnostic evidence a summary statement is possible: Natural infections with the U2-U5 form of TMV occur in *N. glauca* throughout southern California. *N. glauca* is present in many areas, where it has intruded into disturbed vegetation or along the edges of cultivated fields. TMV is most common in bushes beside roads: it is rare but not absent in *N. glauca* away from roads and settled areas. The aucuba mosaic variant, U5, is much less common than milder variants, but it is not restricted in incidence to the UCLA site; the most striking example was in an *N. glauca* bush growing beside a road at the head of the Hemet Valley.

Discussion.—Combined with earlier studies on TMV from *N. glauca* collected in Gibraltar and the Canary Islands (10), our work indicates that the dominance of types related to U5 in naturally infected *N. glauca* is probably common, as dominance of the U1 form appears to be in commercial tobacco. This raises the question whether a group of susceptible plants with broadly similar genetic and somatic make-up may tend to select a particular form of TMV. Differences in selectivity might be anticipated between unrelated groups of plants such as cucurbits (6), legumes (5), and Plantagos (7), but it is interesting that this principle seems to differentiate 2 species, *N. tabacum* and *N. glauca*, within the same genus. There is scattered evidence, also, of similar examples within the *Solanaceae*. Tomato aucuba mosaic has biophysical and chemical properties differing from those of the ordinary severe U1-type of TMV from tobacco (9). The form of TMV that causes tomato streak or stripe disease has reactions on some varieties of tobacco (1) and gives dilution series (4) that link it with U2 and U5 rather than U1. Tomato enation mosaic (2) has corresponding host reactions. A form of TMV from tomato (12) was similarly different from U1. There are other indications in the literature that tomato tends to select a form of TMV differing from that dominant in tobacco.

If the principle of selection or adaptation to the host plant should be demonstrated for TMV by still other examples, the position of ordinary severe TMV from tobacco as the central form from which others may have been derived is seriously undermined. A critical survey of published data on naturally occurring forms of TMV already shows a strong case for the view that the dominant type of TMV in tobacco is a specialized rather than a characteristic form of the virus. This problem will be discussed in another paper.—Department of Plant Pathology, University of California, Los Angeles.

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SYMPOSIUM ON TRAINING FOR THE FUTURE IN PLANT PATHOLOGY
FOUR INVITATIONAL PAPERS PRESENTED AT THE 51ST ANNUAL MEETING OF APS

QUALITATIVE AND QUANTITATIVE ASPECTS OF THE GRADUATE CURRICULUM IN PLANT PATHOLOGY

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I think it is very appropriate that we are, at this time, taking a long look at the problem of training for the future. This year we begin our second 50 years as a society, and I am happy that, at the outset of this second mile, so to speak, we are taking an inventory of our training programs. A few years ago, graduate training in plant pathology in the United States was confined to very few institutions. Training today largely centers in these same institutions, yet many more are now in the business and there are some bright new stars on the horizon. Perhaps the age-old wedding tradition of "something old, something new" is fitting for our situation. The viewpoints of both the old and new are needed. Surely the training of the past has had real meat in it; surely the future requires something new, for a *status quo* philosophy has little compatability with progress.

There was a time when plant pathology had no concern about recruits, but competition today for the talented mind is extremely keen. We cannot have all whom we would choose, and to have even a steady trickle of the best minds we must have a training program that will challenge ability and permit people to discover their own potential. We must have a training program that will equip our recruits to continue to elevate plant pathology among the sciences and further mold our profession in the shape of a basic biological discipline.

I should like us to look at three or four basic aspects of graduate training, and I shall attempt to direct my remarks to these questions. We will be derelict in duty if we fail to realize our problems or refuse to face them.

Specialization in research.—Since plant pathology is primarily a research profession (not in the sense of placing research over teaching or extension in value, but because this is where most of us spend our time), graduate training must, in that sense, become synonymous with research training. Our graduate training must reflect the direction that research is taking or should be taking. One of the most soul-searching problems precipitated by specialization in research and facing training departments is the qualitative nature of the formal course curriculum. To introduce a discussion of this point I should like to raise the question, "Must we at this point specialize or die?"

I tried to find out what some of our early leaders thought about specialization in research and was amazed at some of their viewpoints. Listen to the words of H. A. Harding (PHYTOPATHOLOGY 2: 161-163).

". . . to conserve the host there was developed sprayology in which the aim seemed to be to find a mixture with a new color or a new smell which might be applied several times per year with great profit to all concerned. This has developed to such an extent that at least in the eastern horticultural regions the vernal landscape now rivals the famous Coat of Joseph and the perfume laden air bears the added aroma of Hades.

"The period when specialization in research ran riot and it was fashionable to consider only a minute angle of the problem is passing.

". . . those of us who were victims of the craze for specialization in training, which characterized the past two decades, find ourselves like the original pioneers, poorly equipped for doing work which shall attain the desired standard of excellence."

Here was one who deplored the trend, as he saw it, of studying plant pathology quite exclusively from the host side, as the animal pathologists were doing. Quite obviously, Harding did not espouse the cause of specialized research.

But listen to the words of E. M. Freeman (PHYTOPATHOLOGY 26: 76-82) for the contrary point of view.

"The enormous expansion and increasing complexity of all of the fields of plant science have steadily constricted the scope of operation of the individual scientist. A DeBary, superman in many fields, is no longer humanly possible, and it needs no divine power of prophecy to predict that he will never again appear. Plant pathology of the future must content itself with a growing intensity of specialization."

If we take an objective look at plant pathology in the past 50 years, two things, I think, are obvious.

1) In the early years of plant pathology in this country our people, of necessity, had quite broad responsibilities. A student going out on a job was often expected to work in more than one crop area and with all groups of pathogens. Most of the research was descriptive and with fungi, so *taxonomic mycology was one of the heaviest elements of graduate training.* There was little need for heavy orientation in genetics, because disease resistance and the genetics of pathogenicity were little advanced. Plant pathologists had little acquaintance with a Monroe calculator, and biometry, much used today, was not a part of the early curriculum. Virology and nematology hardly existed as distinct subject areas of plant pathology 30 years ago. They are so much in the ascendancy today that the fungi and bacteria must be blushing from a jealousy of neglect. *Thus, when many of the curricula now on the books were established, the pattern was one of general botanical background, supported by*

some training in chemistry and the emphasis was on mycology.

2) There is now a marked trend toward specialization in research. Gone are the days of the general practitioner, so to speak, when a plant pathologist covered the field, or at least all of a segment of the field. Where we used to have general crop pathologists we now have specialists for specific crops, or even specific diseases of a given crop. Those of us in large training centers notice increasingly that employers of young Ph.D.'s specify research to be done in specific areas such as "physiology of parasitism," "viruses," "systemic fungicides," or "To Join a Team in Crop Improvement," etc. More and more we find ourselves working in restricted subject matter areas. This is, I think, as it should be. It has not resulted primarily from crop economics, or from a design to increase research efficiency, but more from the fact that plant pathologists have come to recognize the multifarious scope of their discipline, and, recognizing it, have come to use the techniques and tools of research of our closely related sciences. They increasingly see and approach problems from different vantage points, such as physiology, cytology, genetics, and biochemistry.

The result is that we have moved and are fast moving away from purely descriptive research and are more and more in basic research. The first 1000 pages of PHYTOPATHOLOGY contain over 90% of what I would call descriptive pathology. It is replete with such titles as "Apple Leaf Spot" and "Notes on Hawaiian Fungi." Volume 47, which had as much printed matter, has much less than 50% descriptive material, and one encounters such titles as "Pectolytic Enzymes Produced by *Sclerotinia sclerotiorum*" and "Altering Disease Resistance With Ionizing Radiation and Growth Substances."

Admitting, then, that we are increasingly specializing in research and that specialization is good, what does this say to us in training centers as to the qualitative nature of the Ph.D. curriculum? Should our Ph.D. degree be based on a broad general curriculum, as in the past, or should we specialize at this level too and train men as virologists, nematologists, physiologists, cytologists, mycologists, bacteriologists, etc.?

I should like to plead for a continuation of a curriculum on a broad, rather than specialized, base. Although we have increasingly specialized, we have not reached the point where most institutions are calling for all specialists. If we permitted sharp specialization at the Ph.D. level, we would undoubtedly have students continuing beyond their degree for one to several years, waiting for an appropriate job. It seems to me that we must follow the medical profession in our approach to this problem and provide specialization of training at the postdoctorate level. What plant pathology needs is an equivalent of the medical residency program, in which the Ph.D. can spend two or three years in a chosen specialty field. This is happening now in many research centers, especially since foundation support is available; and it should increase. This will undoubtedly require a liberalization of leave of absences as it develops. It will mean that students can take advantage of job opportunities that may occur when they receive their degree and can arrange for a leave of absence for a postdoctorate specialized training period, the area of specialization being dictated perhaps by their job responsibilities; or they can undergo such training immediately after receipt of the Ph.D. if no job is available.

Even more important than this, a broad base curriculum also means that one can be a more meaningful part of a research team if one can communicate understandingly with the other specialists of the team. Consider root rots as an example of a disease problem that is complex and requires a team approach. The mycologist could make a much greater contribution if his training also provided him with some understanding of anatomy, physiology, nematology, virology, and vice versa.

Qualitative aspects of the curriculum.—Before considering specific qualitative aspects of a curriculum, I think we might well ask ourselves, "What do we want to wind up with?" or "What do we want our trainee to do?" To answer this question very briefly, I think we could say that we want him to recognize problems, to analyze them, to examine with the tools available to him the component parts of the problem, to synthesize or relate the component parts together again in the total picture, and to communicate his activities to others. This is research. To effect this, our training program should not just provide the factual material a student must have, but should provide it together with a strong discipline of communication and in an atmosphere of stimulation and curiosity. Stimulate him so that he will look and see. Make him curious so that he will analyze and investigate. Polish him so that he can communicate. By communication I mean giving expression to ideas. If the primary purpose of a course is to cram facts into the student's mind, little will be accomplished in the area of communication. If, on the other hand, the improvement of communications is given emphasis, much factual knowledge will be tucked away in the student's mind. One can't give expression to ideas without thinking, and one can't think except in terms of words and facts. Scientists too often do not realize the importance of communication, and consequently place too little emphasis on it. It is a prime factor of success. Marshaling evidence and its articulate presentation are as important to the development of a research field as are experimental techniques. An efficient means of communication often means getting what is needed for the problem at hand or for selling the idea in question. Our courses should therefore be organized and presented in a manner that will evoke maximum student participation in writing, in speaking, and in the give and take of informal discussion. One of the real blights of this day is the increasing size of the class for when the class gets too large, formality and con-

vention creep in until it is difficult to have full participation by the students.

Let us now look at the Ph.D. curriculum, where I think some changes are in order. Even if we keep a broad general curriculum for the Ph.D., it must be expanded, for new areas of training must be added. Traditionally, most departments have given considerable emphasis to "descriptive" or "crops" courses. I plead that we take emphasis from such courses and place it rather on "principles" and on more fundamental courses. Crop courses may be necessary as service courses to crops departments, but surely our graduate curriculum should be of a more fundamental structure. To define what I mean by principles, let me direct your attention to the prophetic words of another of our founding fathers, L. R. Jones (PHYTOPATHOLOGY 1:39-44)—words that have not been generally heeded:

"The advance of plant pathology will be just in proportion to the clear recognition of the fact that its chief problems are biological rather than economic, that the primary concern must be with uncovering of physiologic relations and principles rather than the field trial of fungicides."

If the advance of plant pathology depends on recognition of the basic biological philosophies involved, as I think it does, our training must reflect this. We must deal with principles rather than case histories. Our students must come out of their training period imbued with a philosophy rather than a thumbnail index of plant disease descriptions. They should leave our courses with their minds giving much more play to the words "why" and "how" than to the words "where" and "when." Now, I know many of you would ask, can't a specific course have both? Obviously, the answer is *yes*, but I think most of them do not. They are generally slanted one way or the other.

What changes should we make, and what would constitute a good curriculum in these terms? We should admit at this point that there are many curriculum approaches that might yield very similar products. What I am suggesting merely represents the approach that makes most sense to me. I have studied with care the curricula of our various institutions, and I certainly do not wish to imply that my appreciation covers only the one at the University of Wisconsin. In fact the Wisconsin curriculum of the moment is not what I am outlining below:

1) An undergraduate degree with a major in botany (including a course in bacteriology, a course in mycology, a course in cytology, a course in anatomy, and a course in physiology), and a heavy minor in chemistry and biochemistry. This undergraduate curriculum should include a year of physics, a year of zoology, and as much mathematics, language, and composition as can be worked in. It should include one (and only one) course in plant pathology. In regard to the physics, it is unfortunate that biologists have not persuaded physics departments to present a physics course organized for biologists so that more emphasis can be given to biophysics than to mechanics.

2) The graduate courses for the plant pathology major (and here there are a lot of varying possibilities) might include:

a) A course in principles covering the field but built around basic phenomena. It would provide the student with an introduction to a wide range of disease problems, including both infectious and noninfectious diseases, and examples would be chosen to illustrate basic biological phenomena.

b) A course in methods of research. Considerable increase in classroom time spent in teaching research methods is in order, for no one's research is better than the methods at his disposal. This increase, I think, can be best provided as an integral part of some of the other courses I will mention.

c) A course in the physiology of disease. This would be primarily a course in the physiology of parasitism and disease development, with consideration to both host and pathogen. The laboratory part of it could give emphasis to physiological methods of research. It would, at least for the moment, deal most heavily with fungal and bacterial pathogens.

d) A course in the principles and methods of plant disease control.

e) A course of low credit value in the history of plant pathology.

f) Seminars in contemporary research.

g) A course in plant virology, organized around basic subject-matter areas, with the laboratory portion dealing quite exclusively with research methods.

h) A similar course in plant nematology. This course at most institutions would need to include morphology, taxonomy, and physiology, since no other source for this training would be available. It should also deal with research methods for handling nematodes.

This may seem to constitute somewhat of a dilemma in suggesting basic courses in virology and nematology, with no similar course in mycology or bacteriology. However, this is not necessarily true. Remember that our undergraduate curriculum called for courses in mycology and bacteriology. If these were not provided for at the undergraduate level, then of course they would have to be added to the graduate curriculum. Certainly, we would not be justified in turning out a Ph.D. without this training. Furthermore, the curriculum for the minor, as detailed below, has additional mycology in it. Also the course suggested on Disease Physiology would give emphasis to fungi and bacteria.

There is further justification for special courses in virology and nematology in that these areas have quite different techniques that seem to require special course emphasis. The research methods of bacteriologists and mycologists, on the other hand, are much alike.

3) The graduate courses for a botany minor would include three or four advanced courses in

physiology, including physiology of fungi, mineral nutrition, and metabolism.

4) Supporting courses should include genetics, biometry, and biochemistry.

Students find it a bit more difficult to minor in biochemistry since, with such a minor, one dares sacrifice very little in physiology. Students who are able to carry such a curriculum, however, should be encouraged to do so. Every plant pathologist who has a deep orientation in biochemistry will lift the level of the research around him.

To me the handwriting on the wall clearly says we must apply the principles and methods of physiology and biochemistry to our research. If we do not, others will simply move into the pathology field and be doing our basic research. Our graduate curriculum must simply reflect this and equip our people to do it. If our graduate training is of this basic nature, the research to follow will be of the same kind. If our training is largely descriptive, we will not turn out basic research men. The future of plant pathology as a science lies in basic research, and if we do not chart our course in this direction the crops departments will absorb us.

Quantitative aspects of the curriculum.—The number of courses should be kept to a minimum. Again, this depends on the undergraduate background, and my remarks are based on assuming that there are no deficiencies in the undergraduate curriculum. There must, of course, be minimal standards of formal course training, and it is only time spent beyond this level with which we are concerned. Just as one can invest considerable time in a course beyond the level necessary for an "A," without increasing his grade, so can one take more courses in his graduate training than are necessary to reach his top dividend. The difference in time should be spent in research. Just because a department has a certain number of courses on the books is no reason why every student should take every one of them. Just because a course would be good for a person is not sufficient reason for requiring it or even for offering it. I can't refrain from quoting the words of L. R. Jones (PHYTOPATHOLOGY 1:39-44) in regard to this point.

"While in all this work (meaning course work) the aim is to insure broad and thorough fundamental training, we must beware lest by its very bre..dth and weight we smother rather than stimulate."

Getting an education is a process that never ends, and a student can do most of it after he gets away from his professor. We must be deeply conscious of the fact that one's philosophy of research and work habits often stem from one's graduate training. One can be molded by one's curriculum into an "encyclopedic" pathologist or a strong, well-balanced research pathologist. The number of courses for institutions on a quarter system obviously cannot be the same as for those on a semester system. The thing of importance is the balance of time between course work and research.

The language problem.—So that I will not be misunderstood, let me say something of my training and interest in language. I had two years of high-school Latin. My undergraduate degree was an arts degree, in which I had 30 semester credits in foreign languages. Since these were all elective credits, I cannot be accused of having no interest in, or being afraid of, languages. No one believes more deeply than I in the value of language study. However, there come times when one must assess the relative position of all values, and in this sense I have some things to say about the language requirements for the Ph.D. I believe our language requirements are a farce and should be drastically altered or done away with.

The first problem comes in the choice of language. We are all aware of the heritage our profession has in Germany and France. Many of our forefathers traveled to these countries to study, and 40 or 50 years ago a student of plant pathology had to know these languages to be up-to-date, for the most significant literature was written in them. But now we have had a long period of years in which this is not so. The two world wars drastically altered the pathological literature from these countries. Dutch, Russian, Spanish, or Japanese would seem to be as appropriate for the future. Thus, which one or which two will be chosen, and why?

A second problem has to do with the language standards we require. They do not provide a level of training that results in language proficiency. Students without any course background get a scientific book and begin translating. After three or four failures to pass, they have memorized enough vocabulary and grammar to get through. This would suffice, perhaps, if they made frequent and continued use of their new knowledge, but they don't. The expanse of the literature we now face precludes this. It is simply impossible for us to do what most of us have to do and to read all the literature that is coming out. We have to depend on review journals. This means that we do not use a foreign language enough to maintain proficiency in it, for proficiency in language comes only from knowledge put to frequent use. If our language requirements resulted in proficiency and our use of the language maintained proficiency, I would not for a moment recommend a change. I think we will admit, however, that this aspect of our training is not a success.

What can be done about it? One thing would be to *use* a language enough to remain able to use it. I am afraid this becomes a problem of individual discipline. For most of us it would mean doing less, and we seem to be going in the opposite direction from this. The real answer would be to get our language training at the elementary and secondary school level, or at the latest at the undergraduate level. Until this happens, we would do well to substitute the time used in passing language examinations for more extensive course work in biochemistry or physiology or for a reading course in some allied specialty area such as genetics of microorganisms, medical microbiology, etc.

Miscellaneous considerations.—A) Students should be oriented into research immediately after enrollment in the graduate school. Nothing charges a student faster than to taste success in his own experiments. Nothing can make plant pathology more alive for him than to have an early "baptism of fire" in research. I have seen the basic attitude to research weakened because this does not happen. It is destructive for a professor to think a student should do two years of course work before he wades into research. The good student should be taught to swim early. It is best that the poor one drown at the outset.

B) Something was said earlier this afternoon of the problem of accepting and guiding students of mediocre ability to the Ph.D. Our graduate training program is a part of this problem, and there are some things we can do that will help solve it. In the first place, I think we have made a mistake in America in so completely devaluating the M.S. degree, for in so doing we have inadvertently devalued the Ph.D. We are now at a point where it is almost impossible to find employment for an M.S. student, for the employer immediately figures there is something wrong or the student would have completed the Ph.D. The end is that too many mediocre students get through. Surely there are many places in our profession where an M.S. student can meet the responsibilities of the job at hand. This is one point where European institutions are one up on us, for their research programs have many people of sub-Ph.D. level. I honestly believe we should restore the M.S. degree to a position of meaning and usefulness and that we should strive to use such persons in our profession. This, I hope, would remove the system of some professors' spoonfeeding their students by writing their theses for them, by tutoring them for examinations, or by directing their research too closely. Such training methods are a disservice to the student and to our profession, for nothing develops one faster than being allowed and forced to assume responsibility.

C) I cannot refrain, in closing, from directing one thought to all of us who are teachers, both undergraduate and graduate. I believe that many of us tend to consider research a pleasure, and teaching a chore. How often I hear the expression "teaching takes a lot of my time." If this is our attitude, then our classroom performance will be poor. We simply must not engender this attitude. Teachers should be chosen not only for knowledge but also for their attitude.

Emotionalism is as great a tool of teaching as it is of preaching. Too many teachers enter the classroom without having their emotions at all lifted by the subject matter or by the students. They leave the room just as calm and just as cold. Surely plant pathology is not so dead that it cannot be taught with enthusiasm. Surely a discipline as dynamic as ours should buoy us up to a point where we would glow with enthusiasm, and if the teacher glows, the student will catch fire. If the student catches fire he will have had a great teacher.

RECRUITMENT OF SUPERIOR STUDENTS IN PLANT PATHOLOGY

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No problem now facing plant pathologists is more important than that of assuring a continued supply of superior students, teachers, and researchers in our profession. Only as we attract outstanding young men and women will we be able to advance knowledge in our relatively young science. Our problem of attracting superior students is, of course, a very small segment of the over-all problem of a world shortage of capable scientists. Since the launching of Sputnik, we have come to look upon the shortage in this country as one of the nation's most pressing problems, and many link the dire need for increasing numbers of able scientists in this country to that of survival of the free world.

The recent big emphasis, we all know, has been in the physical sciences, and perhaps correctly so, since they are so directly and practically related to national defense and warfare. Many biologists, however, have expressed alarm, and with good reason, that the biological sciences have suffered in the "big push" toward the physical sciences. I recently asked a noted biologist, "How do we assure the recruitment of superior students in biology–" He replied, "Only when we can match the prestige and economic opportunity of the physical sciences can we in biology obtain our proportionate share of such students." He went on to say that "Today we must recruit graduate students who for the most part have had a much more thorough training in the physical sciences than in the biological sciences." The efforts in the last few years of the American Institute of Biological Sciences toward improving the status and training in the biological sciences are most commendable, and there are hopeful signs of real progress. I shall call specific attention to some of these as I proceed.

I am among those who believe that the great discoveries of the next half century are going to be in biological sciences, just as those of the last fifty years have been in the physical sciences. Certainly man's increasingly profound understanding of the nature of matter will lead to significant discoveries in the complex organization of matter that we call organisms. The spectacular applications of more recent developments in the physical sciences have resulted in their receiving ever-increasing support. There is no question that such support should and will continue to grow as more and more world-shaking events occur. In the midst of these

tremendous advances in the physical sciences, however, mankind must place equal emphasis on his search for knowledge of the basic attributes of life and the biological processes. The ultimate answers to today's practical problems of agriculture and medicine are most certainly rooted in tomorrow's better understanding of the nature of life and its complicated processes. As we in plant pathology press further and further into an understanding of the delicate balance of the nature of parasitism of the organism on the one hand, and the nature of resistance of plants on the other, we are investigating the very basic processes of life itself. Certainly those who will be searching for these answers must have superior training in every respect.

Before a student finally chooses biology, and subsequently plant pathology, as a profession, he must first be sparked somewhere along the line with an interest in science, and in particular the sciences of life and the living. Let us briefly examine the different stages at which young men and women are so stimulated, and the ways and means in which this occurs.

In 1951 Dr. Anne Roe published a most interesting article, "A Psychological Study of Eminent Biologists" (1). She selected 20 biologists in the age group of 38–58 who were among the leaders in their respective fields of biology at that time. One of her objectives was to discover the stimuli involved in the choice of biology as a profession by these successful individuals. It is of real interest to note that only half of these people had a family or childhood background that would have led to an early interest in nature or natural history. This would indicate that a background with a ready access to nature, such as life on a farm or in a small rural area, is not a prerequisite to selection of a career in biology, though it was an important early stimulus for some. Three of the persons studied spoke of gaining an early interest in biology via summer experience on farms, in forests, etc. Certainly, as our country becomes more and more urban, the opportunities for early experience with nature become fewer and fewer for most. The young grade-school student of today is more likely to be sparked by an interest in what makes the television work than in curiosity about how a plant grows. This makes early science experience in the elementary schools, particularly those portions dealing with biology, most vital.

One of the important undertakings of the AIBS, through its Special Curriculum Study Group, is to improve the first contacts with biology that a young person has in the grade school. At this stage, it is my feeling that plant pathology as such should not be stressed, but, rather, we plant pathologists should take every opportunity to further the use of plant pathological exercises in early biology training to illustrate how exciting and dynamic biology can be. As an example, one of our graduate students at Berkeley recently told me that he furnished his wife, who was teaching a 6th-grade science class, with a flat of young safflower plants and a vial of rust spores. The youngsters inoculated the plants, and over the weeks watched the rust disease develop right in the classroom. His wife reported that this was the most meaningful laboratory assignment that the students had experienced until then. Could not our Society's Special Committee on Teaching design a few simple fool-proof laboratory demonstrations for use in biology in the lower grades? These would further the cause of biology and at the same time create an early awareness of the field of plant pathology.

One of the problems with younger children is in stimulating interest in the plant side as well as the animal side of biology. Plant science is often looked upon as static and descriptive, as opposed to the "motility" of animal science. I am sure some of you have had experiences similar to the one I had in this regard. A few years ago, the Agricultural Extension Service asked if I would participate in a 4-H Club conference of some 700 grade-school and junior-high-school boys and girls. They wanted me to prepare an exhibit that would demonstrate in an interesting and exciting manner a segment of plant science. They also told me that there would be a similar exhibit for animal science. The opportunity to show such a crowd of young people some of the fascinating elements that make up plant pathology made me most anxious to have the best possible demonstration. In the preliminary arrangements, I had not been told, however, that the schedule called for the two exhibits to be held simultaneously. When it came time for these, the group leader gave the young 4-H members a choice, asking all those interested in animals to go to the animal barns and those in plants to the plant pathology greenhouse. It is not surprising that I had only about 30 youngsters, as opposed to the some 670 that went to the animal barn. This points up clearly that in very early training it is essential that young people be exposed to all facets of science so that, when the time does come to choose, it can be done with knowledge of the alternatives.

Let us move on now to the high school. Many students at this stage may not have had any experience or real contact with biology, either in their childhood or in primary school. In Dr. Roe's study, referred to above, a number of these eminent biologists looked back to high school as the point where their interest in science first became real. Many pointed to a certain science teacher in high school as having aroused their first real interest in science. Others said that a particularly good course in science in high school had first made them aware of the fact that not everything was known and that science represented an opportunity to create as well as to understand. Thus developed the first awareness of and interest in research. The high-school biology training is of key importance in stimulating potentially good students. The various efforts being made by the AIBS to upgrade the biology offerings in the high school are extremely important, and plant pathologists should give them full support. Too often we have heard a student refer to chemistry

in high school as a solid course and biology as a "non-solid." Biology courses taught as personal hygiene and those taught by persons with no biology training are, of course, "non-solid." Dr. Oswald Tippo, who heads the AIBS Committee on Education, recently said, "As we look back over the educational history of this country, clearly one of the most regrettable developments of the past few decades is the way in which college and university scientists have turned their backs on high school science and the high school teacher" (2). With leaders such as Dr. Tippo, this newly directed attention to high-school biology training will do much toward making high-school biology again a "solid" in the students' eyes.

As far as outstanding high-school teachers are concerned, may I refer again to Dr. Roe's study of eminent biologists, eight of whom said their first interest in science was sparked by a high-school teacher. Seven of those teachers, however, were chemistry teachers, and only one an instructor in biology. This would certainly support the view that chemistry is taught in the high school on a higher plane than is biology, and by better-qualified individuals. It took a high-class chemistry teacher and course to kindle the interest of these seven future eminent biologists. Think of how many more top-flight students might have chosen biology had their first taste of biology been at this level.

As far as high school is concerned, plant pathologists, I feel, must do all they can to support the best possible biology training. We should work toward the inclusion of plant pathology demonstrations and exercises in the high-school biology courses, for these can be some of the most interesting, dynamic, and stimulating in the field of biology. Again may I suggest that the Society's Special Committee on Teaching might look into preparing such exercises for the high-school biology courses. Besides improving the quality of biology taught, they would call attention to the field of plant pathology.

Before leaving the high school, I should like to emphasize the importance of a recent development at some universities by which promising high-school students are introduced to scientific research during the summer. This summer Dr. George Kent described to me such a program at Cornell University, supported by the National Science Foundation, and there are undoubtedly similar programs at other universities. We had working in our plant pathology laboratory at Berkeley this summer a brilliant young high-school boy between his junior and senior years. He is a participant in a program sponsored by a graduate professional chemistry fraternity at Berkeley that has an endowment enabling outstanding high-school juniors and seniors to obtain summer research experience. Our department participated in the program for the first time this year, and we are very enthusiastic about it. Top high-school students, nominated by their teachers, make application to the fraternity and indicate their particular interest. This boy indicated biology, but confessed after a week in our laboratory that he knew nothing of plant pathology and was somewhat worried about his assignment. He now appears to be quite thrilled as he works with Dr. Herbert Gold inoculating plants, assisting in the preparing of virus antisera, etc. He may someday become an accomplished plant pathologist.

Now, finally, let us look at undergraduate college training in plant pathology. We find that many students as they enter college have still not been exposed to any sound training in biology, and almost none have an awareness of or an interest in plant pathology as such.

The four groups of plant pathologists in the University of California, at their last state-wide meeting, in January, 1959, discussed the problem of recruitment and decided to look into the possibility of preparing some type of brochure to call attention to plant pathology. Before doing this, a sub-group headed by Dr. W. B. Hewitt, Davis, felt that we must first ascertain what factors were involved in the choice of plant pathology by the present staff and graduate students. Dr. Hewitt has kindly made available to me the partial returns from a questionnaire designed to obtain this information. The first question asked was at what age did the individual first become aware of the field of plant pathology. Of the 19 responses, only one indicated that he was aware of plant pathology before the age of 18; and 10 of the 19 indicated they were not aware of plant pathology before the age of 20. This would mean the first awareness was in about the junior year in college. Another related question asked at what age did the individual first become interested in plant pathology. Fifteen of the 19 indicated that their real interest in plant pathology began after the age of 21, and 10 of the 19 indicated that interest developed at the age of 23 or later. This would indicate that interest in plant pathology for most individuals developed in the senior year in college or in the first year of graduate work. A third question attempted to determine what factors specifically attracted these individuals to the field of plant pathology. Six of the 19 indicated that a particular teacher, either in high school or in college, stimulated their interest. Nine of the individuals said that a special course helped to create their first interest in plant pathology. In summary, 15 of the 19 recalled that either a special course or a particular teacher sparked their interest in the field.

In these brief remarks I shall consider the recruiting of promising students into plant pathology from the liberal arts college or the small university as a problem separate from that of recruiting students from the various colleges of agriculture. The problem of spotting and attracting into plant pathology the superior students in agricultural colleges should not be as difficult as it is in nonagricultural colleges. First of all, we might assume that many students enrolling in agriculture have had some early and continuing interest in biology (except perhaps the engineers and

economists); and, secondly, in the agricultural colleges there are departments of plant pathology whose members actively participate in the curricula of those colleges and who can personally search for and help to select the superior student. It is from the small liberal arts college that I feel we are missing a great number of potential top-flight future plant pathologists. The majority of the students entering such colleges probably had no early interest in biology, either from childhood or from high-school training. The chances are, further, that most of the students who do enter with an expressed interest in science indicate physics, chemistry, or mathematics as their specific interest. Under such circumstances, it is obvious that the first biology course in such a college is a key one as far as future recruitment is concerned. Many of these small colleges have a single department of biological sciences; others have small separate departments of botany and zoology. Seven of the 20 eminent biologists referred to above pointed to their first college biology course as one that greatly influenced their ultimate choice of a career in biology. For the most part, the first introduction to the field of plant pathology that students have in college is in the beginning botany course, usually under the section on fungi. In a general biology course, there may be little if any mention of plant diseases. In the general introductory botany course, the emphasis is more than likely to be on the fungus and its structure rather than on the disease. Often, the first and only experience a college student may have with plant pathology is in monotonously drawing the five spore stages of stem rust of wheat or in sketching symptoms of peach leaf curl from pickled specimens. I submit that one or two carefully designed laboratory exercises in plant pathology, given in connection with the general botany course or even a general biology course, could present the same information as botanists now teach, but in a more exciting and stimulating manner. It would have the effect of making the first formal contact with plant pathology interesting and might well create in some students a desire to look further into the field. The Society's Teaching Committee might examine this possibility, keeping in mind that in these smaller colleges the likelihood is great that very few of the biology teachers have had much direct experience with plant pathology.

For the advanced biology students in the smaller liberal-arts colleges, very little plant pathology as such is offered. In scanning through a number of catalogs of these schools I found only one listing a course in plant pathology. I found plant pathology mentioned in the description of the following courses: mycology and plant pathology; advanced mycology; study of fungi; advanced botany; advanced research in biology; problems in biology. The opportunity for students to gain experience in plant pathology is lacking because of the small faculty in such schools and the lack of good specimens for study. There is little likelihood that many of these colleges will ever be giving a formal course in plant pathology; in fact, I don't think we should urge this. On the other hand, we should provide for these colleges a number of laboratory exercises that would involve live material, inoculation, isolation, control practices, and many other steps. Such exercises, designed by plant pathologists, could be effectively presented by non-plant pathologists in such schools. They might be part of a course in Advanced Botany, or one on Special Problems or in Research in Biology. Such contact with plant pathology by students already interested in biology might well convince them of the advantage of trying graduate work in our field. The visiting-scientists program that the AIBS has initiated with such colleges is extremely important in this regard, and we are all pleased to know that some of our most enthusiastic and inspiring fellow plant pathologists are participating in the program. It is also important that the departments of plant pathology throughout the country keep in close touch with the botany and biology departments in these colleges. We should assist them in every way possible with teaching materials and laboratory outlines, provide ways for their students to obtain summer research work in our laboratories, and, of course, continually express our interest in their better students.

Turn now to the agricultural college. Here again we must try to make the student's first experience with a plant disease in a general botany course as meaningful and stimulating as possible. As I suggested above, the botanist might consider using one or two laboratory exercises designed by a plant pathologist to illustrate the botany he is teaching. There may be the opportunity in these universities, which have a college of agriculture, for plant pathologists to participate actively in the one or two general botany laboratories dealing with plant disease.

The introductory course in plant pathology offered at the undergraduate level in agricultural colleges is one we are all vitally interested in. If taught well, it will unquestionably create a wish to learn more about plant pathology. It is this course that our Special Teaching Committee is examining carefully. I feel that this effort can be of great value to the plant pathologists who are teaching these courses—particularly in that it will provide new ideas and approaches for laboratory exercises. As I have repeatedly stressed, however, the great contribution of this committee will, I feel, be in designing a few laboratory exercises that can be utilized in the beginning botany courses in both the agricultural and small liberal-arts schools and a series of other exercises that can be utilized by non-plant pathologists in the advanced biology training in the small non-agricultural colleges.

There is some disagreement concerning the necessity or even advisability of offering an undergraduate major in plant pathology. There is no question that many of our fine students and current leaders in the profession took plant pathology as an undergraduate major. In the planning of today's curricula, however,

we have the continual tug of war between the increasing specialization of science, which some feel calls for more and more courses, as opposed to those urging the necessity of a greater amount of liberal arts and humanities for the future scientist in his training. I submit that these goals can both be achieved, but not in the undergraduate curricula. I feel we should strive for an ever-increasing broadening of the undergraduate experience of the student, with specialization coming almost entirely in graduate training. An undergraduate major in plant pathology tends to deprive the student of a broad undergraduate experience and also creates the danger of building the superstructure before the foundation is completed. Perhaps an undergraduate major in plant pathology, if there is to be one, should be designed to prepare a student at the end of his undergraduate course for a specific type of endeavor, such as crop protection.

In conclusion, I should like to stress the following points as I see the problem:

1) We must realize that what stimulates one person may not stimulate another, and thus we must pay attention to all stages, the elementary school, the high school, and the college, in our effort to attract more and more superior students into plant pathology.

2) We must give full-fledged support to the AIBS in its magnificent efforts to up-grade training in biology.

3) As plant pathologists, we should endeavor wherever possible to utilize plant pathology as one of the best examples for vivid illustration of biology to students and citizens alike.

4) Our teaching committee should consider the advisability of preparing laboratory demonstrations, exhibits, and exercises for general-science, biology, and botany courses at all stages from the elementary school through the college.

5) In our continued search for superior students, we should pay special attention to the small liberal-arts colleges and the smaller nonagricultural universities, from which I feel we have yet to attract our share of potential top-flight students and future teachers in plant pathology.—Department of Plant Pathology, University of California, Berkeley 4, California.

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IMPROVING THE INTRODUCTORY COURSES IN PLANT PATHOLOGY

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Some of you will doubtless agree with the late Robert Benchley, who is credited with the adage that "too much wisdom gets on the wise man's nerves." Others may well fear that our present exercise stands in danger of becoming, to paraphrase a recent writer in the Montreal *Star*, one of those adult educational get-togethers at which serious men lecture mainly to the converted! Perhaps plant pathologists can be trusted to eschew either of these extremes and at the same time examine with profit some of the grave problems confronting us in the matter of education. I shall attempt, therefore, to be both brief and informal —there is certainly little danger that I shall be overwise.

Dr. Oswald has just spoken to you, and Dr. Sill has commented, on superior students and teachers in plant pathology. If we can assume that "superior" means what it did to the United States Army ten to fifteen years ago—most of you will recall a rating system that ranged from "satisfactory," at the bottom, through "very satisfactory" and "excellent" to "superior" (reminiscent of the fruit grading system attributed to our California colleagues, i.e., "large, larger, giant, jumbo, colossal, and supercolossal")—if "superior" means this, then we have the answer already and the remainder of our symposium is without point. Given "superior" students, it makes little difference how we teach, we cannot prevent them from learning. Assuming superior teachers, we need not concern ourselves with methods, materials, manuals, or measurements. If we had both, plant pathology would certainly be civilization's most exclusive and honored profession.

But we must be realistic. Too many of our students leave much to be desired; too many of us who teach are all too ordinary. The total reservoir of that most precious commodity, human intellect, is all too small. We must never relax our efforts to achieve "superior" ratings for ourselves, and to recruit "superior" students, but we cannot in the meantime ignore an obligation to get maximum yield with such material as we now have. The title of my talk might more properly read "Improving mediocre introductory courses in plant pathology for run-of-the-mine students." It is with this sort of course that I am myself involved and within this context that I proceed.

I know of no all-inclusive solution to the problem, nor do I believe that there is one. Very likely it will require our best efforts in a number of directions—background training for the student, textbooks, audio-visual aids, facilities, instruments and equipment, course content and curricula—if we are to achieve optimum results. I should like to suggest one step in what seems to me the right direction, and to solicit your comments, criticisms, and alternative viewpoints. Surely, more will be accomplished by a full and critical examination of one item than by a superficial treatment of several.

It is particularly appropriate and necessary that this suggestion be considered here, for a number of preparatory steps have already been taken. The project is already under way. It has been considered and approved by the Council of the Society and by the Teaching Committee. We have a grant of funds from the National Science Foundation and have selected a planning committee. Finally, I might add, the program has been roundly damned by several of our more eminent colleagues—its success is therefore virtually assured. I refer in these remarks to the anticipated preparation of a Sourcebook of Laboratory and Field Exercises in Plant Pathology.

Perhaps four years ago, the Division of Biology and Agriculture, of the National Research Council (which is not the National Science Foundation, by the way, and is not a governmental agency), undertook a major enterprise designed to produce a series of sourcebooks in the biological sciences. These were planned as collections, screened from a large mass of contributed material, of laboratory and field exercises, each compilation to contain far more items than could possibly be incorporated into a single course, and from which individual teachers were free to pick and choose as they saw fit.

To obtain material, a very large initial solicitation (12,000 if I recall correctly) was sent out, addressed to most of the nation's teaching biologists. The brochure explained the program in detail, the circumstances of its origin, and so on. Many of you must have received this communication—some few may even have opened it and studied the contents.

In the original plan, there were to have been a series of possibly eighteen such compilations, beginning with secondary school biology, then college biology, and on to the several fields and specialties. One of these latter was, of course, plant pathology. But to make a very long, very difficult, and very expensive story short, a number of fine exercises were submitted for the high school volume, a fair assortment for college biology, and relatively little in the specialties. I recall that there were *two* items in plant pathology. At the present time the secondary school sourcebook, which was prepared during an eight-week writing conference by a group of thirty high school and college biology teachers, is in mimeographed form (6). It has been received with unreserved enthusiasm by those teachers who tried it out last year, is now being revised in the light of their comments by the senior editor, Dr. Chester Lawson, and a contract for the commercial production of the book has been negotiated. I can vouch for the success of this book and for the fact that it has more than repaid the time and money spent thus far. And just to close this chapter of the story, three additional volumes are still in the mill: 1) the college general biology sourcebook is being further processed by the college level participants in the 1957 writing conference; 2) the American Physiological Society has undertaken the task of working up material submitted in that area; and 3) the Society of American Bacteriologists has received the microbiological items.

If I may now express a personal conviction as if it were indeed fact, I would insist that the failure in solicitation of plant pathology exercises—and this is doubtless the case in other areas—lay not in lack of interest of the scientists concerned with teaching, nor in a paucity of worthwhile material for inclusion. It was a failure of technique. You simply do not approach a few hundred specialists with the same broadcast appeal as a half-score thousand general biology teachers. I have therefore urged that the Society itself, through its Teaching Committee, undertake the preparation of a sourcebook along the general lines of the present high school volume, but by a very different technique—namely, by concentrating on a person-to-person contact among teaching pathologists.

The project as we submitted it several months ago to the National Science Foundation stipulated a planning conference of several days (scheduled to follow these meetings immediately) wherein twelve individuals (C. W. Boothroyd, B. H. Davis, I. W. Deep, S. Diachun, J. P. Fulton, J. W. Hendrix, A. Kelman, J. E. Mitchell, J. H. Owen, W. H. Sill, Jr., R. B. Stevens, and R. Zabel) actively involved in introductory teaching could not only establish procedures for collecting material and preparing the sourcebook but debate general issues and objectives pertinent to plant-pathology education. Following this, a year or so is to be devoted to assembling material by personal solicitation among interested colleagues, and, terminating that, a three-week writing conference of six persons to handle editorial matters and prepare the final manuscript. The program is based on the convictions that: 1) there is, in the biological community as a whole, a considerable number and diversity of interesting and worthwhile laboratory and field exercises already in use, but that many of the best of these are known only to the person who originated them and to his immediate students; 2) most individuals would be happy to have their ideas more widely used if some ready means of dissemination could be devised; and 3) if the number of exercises and field studies incorporated into the final volume were great enough and if they were adequately varied and diverse, the individual teacher could select those adaptable to his own course. To this I would add my own feeling that the very best material is likely to come from efforts to transfer useful and provocative items from the research field to the teaching field by adapting appropriate research activities or projects into laboratory exercises. In my own teaching experience, certainly, students responded best to the exercises that I fashioned from research actually being carried out in colleagues' laboratories. Where possible, of course, a candidate exercise of this kind will be tried out in the teaching laboratory before being included in the sourcebook.

A glance at our agenda for the planning conferences would show that we propose to begin this con-

ference with a discussion of principles. I believe this is entirely appropriate, and indeed it interests me more than any other single aspect of the program. If I were asked what one thing would contribute most to "improving introductory courses in plant pathology," it would be an increased attention to, search for, and emphasis on, conceptualization and generalizations (as distinct, of course, from generalities). I submit that chemistry suffers in contrast with physics, biology in contrast with chemistry, and the agricultural sciences *vis a vis* other aspects of the life sciences, in almost direct proportion to the extent to which they have failed of conceptualization. If we are to compete effectively for manpower and support, if our science is to attract its fair share of the truly gifted, we must show more interest in these special challenges. If we are to make optimum use of such personnel and material resources as we now enjoy, we must bring the search for valid generalizations to the forefront and make this search a respectable endeavor. For just so long as we present our science as an accumulating mass of information, while the physical scientist presents his as an exciting intellectual endeavor—for just that long we will not attain the levels we should like to reach.

But there are special problems. I am all too acutely aware how difficult our task is. To conceptualize plant pathology and thus raise it to the level of a science is not simply to copy the techniques of the physicists—by comparison with the obstacles we face their problem is simplicity itself. I say "simple," not "easy"—the two are quite distinct.

Much could, and probably ought to, be said on this topic. I shall have to content myself here with calling to your attention five fairly recent events that seem to me pertinent—five straws in the wind, so I would like to think. In three of them I was privileged to participate; of two I can speak only indirectly.

1) In October of 1955, thirteen biologists representing a wide range of special aspects of the field, met in Lee, Massachusetts, at the so-called Conference on Concepts in Biology. Here they discussed the status of conceptual thinking in biology, whether indeed biology can and should stress this approach in the same sense as in other fields of science, and, if so, what can be done to give impetus to the effort. A full report, largely in dialogue form, has recently been published (2).

2) Somewhat later the Committee on Educational Policies, Division of Biology and Agriculture, NAS-NRC, convened a two-part conference on undergraduate curricula in the biological sciences to consider three problems: a) Should an acquaintance with biological science be expected of all college graduates, and what objectives, characteristics, and content are recommended for introductory courses? b) Beyond the introductory program, what core of biological and other knowledge can be recommended for all future biologists? and c) What is the role of undergraduate work in preparing students for different biological specialties? A careful reading of the report of this conference (5) will show, as was so apparent during the discussions themselves, a laudable concern with biological principles as the core of the undergraduate curriculum.

3) As a pilot operation, the Division of Biology and Agriculture set up two panels with instructions to develop sample outlines for college courses in the principles of parasitism (4) and in systematic botany (1). While I personally much prefer the result of the second panel and was disappointed that the parasitism group largely overlooked the plant parasites, each outline stressed what I think may fairly be called a "principles" approach—using the term here in a thoroughly commendatory sense.

4) It is particularly encouraging to note that the National Science Foundation, for the first time this summer, has seen fit to support an institute on the History and Philosophy of Science and Mathematics. You are familiar, naturally, with their very extensive series of subject-matter institutes, but in the instance just noted we have evidence of a new concern for the philosophical framework of science. In fairness I must point out that biology got but scant attention by contrast with the physical sciences, but for the present that is what we have every reason to expect.

5) Finally, and just in passing, I should remind you of the very ambitious curriculum study launched by the AIBS in Boulder, Colorado (3). It is too early to judge whether they will give any thought to conceptual matters, but they certainly have a magnificent opportunity to do so and it is to be hoped that they will.

While only a random sample, in the aggregate these events seem to indicate an exceedingly healthy interest in trying to do for the biological sciences what has long since been accomplished to a large extent for the physical sciences. This is not to say that the biologists have been heedlessly negligent. Rather, in the hierarchy of material systems, in the levels of complexity encountered in the material world, it is both necessary and inevitable that mathematics shall precede physics; physics precede chemistry, chemistry precede biology, and biology precede the social sciences—or, perhaps better, the science of societies. Too bad they are not taught in this sequence, incidentally.

Nor is it to say that the concepts of physics, once arrived at, are immutable and unchanged. Quite the contrary, they have been forced to undergo at least two major upheavals in less than three centuries. This is as it should be, and physics is the better for it. I hope I shall never be found arguing for the formulation of a rigid set of concepts in any field, however persistently I may argue for what I consider the rightful place of efforts directed toward the formulation of useful generalizations. This certainly must have become clear by now.

Reduced, then, to categorical statements, it seems to me we face these problems:

1) Can biology, with its special problems and its

peculiar complexities, hope to have a conceptual framework roughly comparable to that of the physical sciences?

2) Assuming, and I think justifiably, that biology can be conceptualized, does the clearly synthetic nature of a discipline such as plant pathology either a) affect the utility of biological concepts as applied to that discipline, or b) introduce any concepts unique to it by virtue of its special nature? In short, are biological concepts transferrable, valid, and useful for plant pathology, and does plant pathology have any special concepts or corollaries of its own?

3) Assuming that the answer to Question 2 is generally, or even in part, affirmative, what are the concepts of plant pathology, how do we go about discovering them and enunciating them, and how do we make their formulation, consideration, and review a generally respected endeavor?

4) How do we introduce these generalizations, and, much more important, enthusiasm for this particular activity, into our teaching process?

It is you, and your colleagues, who must help answer these questions. I shall be making my own suggestions. I have tried to outline one step we hope to take in this direction, i.e., to cast the details of our sourcebook into a semblance of this mold. Of only one thing I am completely certain: our introductory course can become what it should be only if we cease to teach about "plant diseases" and concentrate on "plant disease."

And while we are about it, let us find some way of getting the point of view and the special challenges of plant pathology into the teaching of other biological science courses. I think it little short of disgraceful to find high school and college biology, introductory botany, indeed even plant ecology, plant physiology, and parasitology, almost completely devoid of any consideration of disease in plants as a biological phenomenon. Both we, and they, are the losers for it.

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THE TRAINING OF STUDENTS FOR RESEARCH IN PLANT PATHOLOGY

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Many experienced persons will argue that gifted scientists are born but never made. There is a measure of truth in the idea, but it is fallacious to assume that men's natural aptitudes for research cannot be improved by proper training. The type of training, of course, must be varied according to the innate abilities of the student. Anyone who works with graduate students very long comes to recognize that they fall into three rough categories: the gifted, the promising, and the difficult.

Some students in each generation seem to be most generously endowed with the aptitudes essential for research: driving intellectual curiosity, devotion to duty, precision in experimentation, persistent and sustained effort in the search for truth, and sound, objective judgment in evaluating the significance of their observations. When these attributes are well balanced, the man is a gifted candidate for a career in research. Nine times out of ten he will become a leader, regardless of how crudely he is guided through his graduate training. Such persons should be taught the fundamental rules of scientific investigation and be given freedom to grow. About all the professor needs to do is surround them with sources of knowledge, teach them techniques, and expose them to provocative ideas for investigation.

These gifted natural leaders, however, are so few that the multitude of pressing problems in plant pathology can never be solved by them alone. They can spearhead the effort, but this science will stand or fall on the productivity of the good, solid scholars who are less gifted with creative imagination. These wheelhorses of plant pathology must be trained carefully so they become competent artisans of science. Once they are exposed skillfully to the vision of the great problems still to be solved and the exhilarating pleasure that comes from blazing new trails into a wilderness of ignorance, they can be inspired to a productive life. It is toward this group that major effort should be directed in training students in the technology of research procedures.

The third type of student fails to meet the minimal prerequisites for a promising career in research.

First and foremost, he may not have ability to acquire a relatively large volume of usable knowledge. No one knows how creative ideas are born, but they certainly come from people who are able to master facts and reshuffle them into new relationships to each other, thereby becoming amenable to new interpretation. From these new interpretations, hypotheses can be promulgated and new principles established. This attribute implies a burning curiosity regarding the basic principles of natural phenomena and a desire to acquire new facts wherever they exist in books,

in other people's words, or in astute personal observations of natural phenomena.

Secondly, the student may not have tenacity of purpose and resourcefulness. Research is nothing more than a process of self-education, in which the acquisition of knowledge is projected far beyond the security of the printed page or the kindly guidance of a learned professor. There are no sure charts that will prevent the researcher from taking the wrong fork in the mental road or plunging into a morass of confusion over his head and even beyond the present status of knowledge. He must be willing to retrace his steps and try again and again to bring logic out of confusion.

Finally, he may not have real integrity. He must be unwaveringly honest to his data, to the public, and to himself. Science is the search for truth, and nothing less than full truth can be accepted. Facts must be faced fairly and squarely, no matter how devastating they may be to pet ideas and personal hypotheses. Research integrity calls for more than one or two positive facts; it calls for the best effort to disprove an idea before it is promoted as factual knowledge.

If a candidate is seriously lacking in any one of these three basic attributes, it is doubtful whether he is worth spending the effort of training him for research. He probably should be diverted into a career along some other lines. However, if he is not deficient in these basic requirements, there are certain sensible rules and guideposts for training him to become a substantial and serviceable research man. Unfortunately, the methods now employed in teaching graduate students for careers in research leave much to be desired. It is time for the teachers to look at some of the fundamental mistakes we have been making in guiding young people.

Training a man for research is much more than a matter of exposing him to knowledge. It is a process of arming him with precision in thinking and creative imagination, two attributes that all too often are sorely mangled by 16 years of indifferent and uninspired education before he gets to graduate school.

The best that the graduate experience can provide is a reorientation of his thinking so he will search for basic principles. This calls for reorganizing his formal courses so that they stress principles, and seeking research problems for him that project ideas from known principles into new areas of investigation. The actual training must stress the prerequisites of good research: methods of solving problems, choice of a significant problem for investigation, precise application of research skills or techniques, and sound judgment in appraising progress. It is to these four areas that we should direct our attention in teaching at the graduate level for careers in research.

The first principle, problem solving.—A research man has one function to perform. He must be able to solve problems. There is no need to confuse the issue by discussing pure or basic research vs. applied research or to try to define the kind of project outlines he will be expected to follow. His job from the day he enters the profession until he retires is to solve problems. The tasks may vary in magnitude and character, but they always come back to problem solution.

Effective solution of problems has two basic prerequisites: knowledge of the nature of the problem to be solved, and discovery of a way to tackle it. The only way to approach the problem is to ask questions. If the researcher knows how to ask himself the proper questions, he is almost certain to find proper answers. The proper phrasing of the question is basic to initiating a research effort.

Please note that the first requirement of a good research man is ability to apply himself mentally to a tough task. He does not start out with a technique to do something in the hope that he will learn something that is new. The latter is like the winding pathway of a meandering cow that hopes a green blade of grass lies over yonder hill. This is not research, yet at least 80 per cent of our graduate problems are launched from this very fallacious viewpoint.

The young person who is trained to do research by routine application of a few techniques soon finds his mental horizons bounded by those manipulations. He has limited capabilities and often lacks the depth of perception and breadth of purpose that will encourage him to attack a big problem and solve it by whatever means are necessary for its solution.

Therefore, the first principle for training a research man is to implant in his mind that he is to solve problems. A great deal of the difficulty in keeping plant pathology progressive has arisen from the failure to teach this basic principle. For example, how many departments today are teaching research as a direct application of Louis Pasteur's pure-culture techniques and Robert Koch's postulates for the proof of parasitism? For over 70 years this *was* plant pathology to generation after generation of plant pathologists. It left them deaf to the greater opportunities knocking on the door. Of course, every neophyte must learn pure-culture and inoculation techniques, but they are nothing more than tools for solving problems and not an end in themselves. There are still diseases to be identified, but the greater area of service lies elsewhere.

The ecological aspects of disease are crucially important, but how many hundred theses have included the routine growth-temperature curve of fungus "X"? One unkind, but very wise, viewer of the scene once told me that at a certain graduate school in this land all one had to do was change the name of the fungus in the title, insert a new set of numbers in the columns marked "°C" or "% RH," and one would have a new thesis, paragraph by paragraph. Obviously, the professors were not solving problems; they were using a routine technique over and over. We fully realize that humidity cabinets and temperature tanks must be used after they are bought, but if research thinking stops at their doors, the future of the science is bleak.

The curse of unimaginative guidance in graduate school has a devastating effect on young scientists.

Over the past 15 years it has been my pleasure to interview several-score of young scientists fresh from graduate schools, in either plant pathology or related professional disciplines. When these men were asked exactly what sort of research they would like to do if no pressure were brought to bear by the employer, most of them were utterly confused. They were apparently bewildered at the idea of having to think for themselves or to dream of the problems that should be solved. A few, thank God, recovered, and their eyes sparkled as they defined a field. Now and then, one had a vision of a greater service to be performed, but most wanted to dig a deeper and wider rut exactly where their professors had placed their feet. What is wrong with a system that sends out bright young men without motives or creative desire?

They actually want to be told what to do and how to do it. Unfortunately, this is the one thing that management in a good research organization should not do. As you all know, research managers are not supposed to be very smart. They must find a creative man (or woman) and needle him into living up to his full capabilities. They are obligated to find money and tools of the trade, but from that point on the research man should take over—not only to carry out the assignment but to fight for the new ideas he would like to develop.

The second principle, choice of a problem.—It is not enough for a young man to be imbued with the idea of solving a problem. He must also learn how to choose a worthwhile problem. As mentioned, he should religiously avoid repeating another man's experiment by mere substitution of another fungus, bacterium, or virus in an established experimental setup.

There is substantial need for good solid workmen who will fill in the finer details after others have set the pattern. There is no reason to be ashamed of doing this as new diseases come upon the scene. Such research, however, does not challenge creativeness in the individual, and he may operate far below the level of maximum production. Somehow the student must be imbued to seek a fresh approach to old problems and to be keenly alert to the solution of new ones.

Are our courses organized so as to encourage such creativeness? Let us examine them as they are run off in the ordinary classroom. The first thing the students are told is that we will discuss, in order, the diseases caused by bacteria, phycomycetes, rusts, viruses, nematodes, etc. The course drones on through the bacterial soft rots to crown gall, and thence, as likely as not, to wilt of cucurbits to angular leaf spot of cotton. Six months later they may pass by Rhizopus rot of strawberry or wound tumor virus of clover without seeing the natural pathological relations.

They have been taught mycology, etc., with plant-disease specialization or implications, but they do not know much about the nature of disease. Consequently, when they graduate and begin research they look at disease as a mycological phenomenon. The functional aspects of phytopathogenesis have escaped their attention. The only better method of being certain to obscure the basic processes of disease production is to group the diseases by crops. We are fully aware that plant pathology must be taught as a service course in the Land-Grant colleges. This is all right as long as it is recognized as serving a technical need much as agricultural engineering does, but it is not the pathway for serving the professional student of plant pathology who needs to understand basic principles.

The more effective way of training future research men is to emphasize pathogenesis. How does the pathogen create disease? What does the host do in its reaction to invasion, and, eventually, what sort of a metabolic pattern develops from the pathogenic state in which the host and parasite form a living entity often approaching commensalism? Such a system of teaching stresses invasiveness and counteraction so the student sees the real problem of disease. It is no longer an isolated pathogen or a host plant injured, but a new state of symbiosis.

The need for basic research is suddenly brought into focus very sharply by such teaching. The pathways that could be explored in controlling the disease, for example, are automatically obvious. Thus, the choice of research problems can be made very simple if the student has been taught to think in terms of fundamental facts.

The fundamental facts are very simple. The primary fact in all plant pathology is the one of nutrition or nourishment of the pathogen. The metabolism of the host is altered by the pathogen, either by inroads on food supplies or by the production of biologically active compounds that alter the host metabolism. Parasite action and host reaction are basic considerations in studying pathogenesis.

The investigator who has been taught to look upon disease as a dynamic physiological process will have his feet oriented into pathways of creativeness where his lack of imagination will be the only possible barrier to progress. This viewpoint is difficult to teach because most of the facts are missing. But look how much better off we are than at the Pittsburgh meetings in 1948, when the writer first pointed out the need for a more basic approach to the teaching of plant pathology.

In the intervening years we have seen the very basic investigations on the metabolic activities of the wilt fungi at Zurich, Connecticut, and Wisconsin. We have had the good fortune to see Dr. Gaumann direct his energies in scholarly interpretation from mycological considerations toward what he prefers to call plant toxins.

Plant pathology has a unique contribution to make toward all aspects of science by pursuing such research. Witness the discovery of a new amino acid as the toxin of wildfire of tobacco and the groundswell of activity in plant growth regulation research from the discovery of gibberellic acid in the causative complex of the bakane disease of rice. But why have plant pathologists been so slow to exploit such gold mines

of information? Obviously our minds have not been sharply focused on basic principles.

There has been developed during the past decade, a greater understanding of the role of nucleic acid in regulating the function of host cells infected with viruses. The metabolism of viruses in the host has begun to be explored. We find the younger generation of scholars making the first tentative steps into questions of protein and amino acid nutrition of diseased plants, as witnessed by several papers at the 1959 meetings of the American Phytopathological Society.

This willingness to look beyond the superficialities of host and parasite relations is most encouraging. It means that graduate schools must train their young people soundly in biochemistry, physics, mathematics, and organic chemistry. The future for such people should be bright in plant pathology research. Ahead of them will be found virgin and profitable areas of research. Some of those areas are: the hormone activity of metabolic products of pathogens, the antagonisms of soil microorganisms for plant pathogens, the effect of root secretions of plants on establishment of parasites, the mechanism of fungicidal activity, the biochemical processes of host resistance, the regulation of leaf and fruit abscission, the chemistry of abnormal proliferation of cells, and the basic processes of obligate parasitism.

Plant pathologists of this generation have their own carelessness and lack of foresight to thank for several failures. Because we refused to look into the biochemistry of disease processes we failed in many ways to achieve greater areas of service. When one appraises what should be done for the future, it does not hurt to look backward to see wherein we failed from time to time.

We failed to explore antibiosis in the soil vigorously. Fleming's observation on penicillin followed comparable observations on bacteria by Erwin F. Smith, in 1916. The extraction of fungitoxins from fungus cultures by the writer and Weindling's observations on gliotoxin action in 1932 revealed that this era of antibiosis was at hand, but plant pathologists were not chemically inclined. The antagonism of soil microorganisms for plant pathogens was well known, but the antibiotic field went by default to the bacteriologist. The effects of *Gibberella fujikuroi* were well catalogued, but chemists had to identify gibberellic acid and physiologists developed its use. In each case, opportunity was knocking on our door to study disease processes, and we ignored it. The opportunities for choosing such worthwhile problems are still with us but they will not be exploited fully until students are trained to seek the right answers to pathogenesis.

The third principle, developing research skills.—If a research man has located a real problem worthy of trying his mettle and has developed some ideas that might solve that problem, he must then put the ideas to experimental tests. The type of experiments he will design will depend on how well he is trained. If he has at his command a diversified group of skills, he will be able to draw on many techniques to solve the problem. If his training has been restricted—for example, to running field trials with fungicides—he will avoid an experiment on respirometry, which would be far more useful. On the other hand, if he has spent all his time working in respirometry, he may be hesitant to attempt separation of organic acids by elution-column chromatography. Consequently, his reasoning powers and mental processes are automatically circumscribed by limitations in manipulation.

Such a person must be ready to team up with someone else who does have these skills, or he must be adventuresome enough to strike out on his own to master the skill from the literature. The latter is a hard and often discouraging pathway, but it is the trademark of a good scientific workman. However, the person who has the responsibility of designing courses in plant pathology has the responsibility of introducing the student to as many research tools and devices as possible and to make him aware of sources of information on the other techniques.

The day of plant pathology based solely upon a transfer needle, Petri dish, and hypodermic syringe is dead. Of course, every student must be grounded in the fundamentals of pure-culture and inoculation techniques for bacteria, viruses, nematodes, saprophytic fungi, and obligate parasites. To these must be added the ecological skills of soil environment control and air condition control. He must be given the standard techniques in physiology on such things as regulating soil nutrients and studying plant responses, such as growth rates, respiratory mechanisms, auxin analysis, transpiration forces, food transfer, etc. These things ordinarily are covered fairly well in the better graduate curriculum.

The thing that is often entirely missing or at least woefully inadequate is the training in the operation of instruments of biochemistry and physical chemistry that is so essential to the study of chemical behavior. These techniques are relatively simple and easily taught. Among them are such things as paper chromatography, column-elution chromatography, ultraviolet spectrophotometry, infrared spectrophotometry, high-speed centrifugation, electrophoresis by Tiselius and paper migration techniques, use of radioactive tracers, etc. The use of these devices brings chemical analysis and chemical thinking down to very simple terms, provided the student is reasonably aware of the pitfalls involved.

It would seem that every good curriculum in plant pathology should include a good course on techniques to augment the routine skills learned in ordinary laboratory courses. The plant pathologist will have to teach many of these skills since the student simply will not have enough time to take all the courses available in chemistry, bacteriology, physics, etc., where the various types of instrumentation and the theories behind them are covered. This means that the professors must first master the skills for themselves even if it does mean they must step down to

the role of being students before aspiring to direct their own proteges.

Developing sound judgment.—Common sense and ability to appraise progress objectively are God-given attributes of some men. However, the investigator can be armed with proper values and techniques for critically appraising the value of data. Some of the claims being fostered off on plant pathology today as fact or "reasonable ideas from the facts at hand" are so poorly substantiated that they are a disgrace to the profession.

Students must have ingrained into their thinking the moral values and responsibilities attendant to their station in life. The professors must make everyone realize the terrible weight of all sins of omission just as much as the disgrace of commission of acts of error or deceit. Every idea should be tested from every conceivable angle before it is released. Never should there be any shade of carelessness in advancing ideas as facts before the weight of evidence is completely overbalanced in their favor. This is the code of morality or ethics in plant pathology that has been mangled disgracefully by improperly trained students who have never become scholars. Many start practicing the loose and easy approach early in their career and never do recover. They continue even in maturity to live on their cute ideas rather than on solid facts.

The elementary laws of logic must be used in every problem so both the forces of inductive and deductive reasoning are applied. Analogy must be looked upon as the poison or opiate of the scholar. It is never enough to test an idea by one single line of reasoning or research approach. Not only must logic be sound, but it must be exercised from as many basic premises as possible. Every tool and device must be exercised *to try to disprove the idea* one cherishes the most.

In addition to wise use of logic, every experiment must be looked at for treacherous chance and miscarriage. The data coming therefrom must be analyzed logically and subjected to statistical analysis. A quantitative and objective manner of taking data must be set up and adhered to religiously. Then the data must stand or fall according to statistical standards.

The gain during the past quarter century in statistical manipulation of data to determine confidence levels, correlation coefficients, and factorial analysis is almost unbelievable. No student can be considered adequately trained until he comes to have a complete understanding of the demands of statistical logic and is willing to subject all his data to their critique.

Finally, many young scientists are coming out of school today without an inborn desire, or complete understanding, of the need of repetition of each and every experiment that is believed to have contributed a new facet of information. The honest facts are that human beings are prone to make errors. Neither all the statistics in the world nor precision of instrumentation will completely remove the error of plain foolishness and human frailty. Furthermore, there simply are too many unknown factors in every biological system for anyone to be complacently sure that he has all factors under complete surveillance and control. Repetition in season and in space is imperative before arriving at broad general conclusions.

The challenge.—Plant pathology has been a very progressive branch of botany since its inception on a sound scientific basis, almost 100 years ago. During this time magnificent progress was made in determining the causes of various diseases and in studying the nature of the etiological agents. Much knowledge was acquired on the effect of environment on the pathogens and their interrelationship with other organisms.

In this country, the profession has been very fortunate to have had many progressive leaders in research. Among the early architects were such giants as L. R. Jones, Erwin F. Smith, J. C. Arthur, H. H. Whetzel, and W. A. Orton. The list of senior statesmen of today includes such men as E. C. Stakman, L. O. Kunkel, J. C. Walker, I. E. Melhus, C. W. Edgerton, and George W. Keitt. History probably will have a high regard for latter-day disciples such as J. J. Christensen, George W. Fischer, Armin C. Braun, James G. Horsfall, K. Starr Chester, W. J. Zaumeyer, and Lindsay M. Black, in spite of any human frailties they possess. These men and many others like them have blazed the way into new areas of knowledge and given plant pathology a firm foundation on many frontiers.

The problem in training the next generation for research is to inspire them by these examples without creating such an attitude of hero worship that they will want to follow in exactly the same footsteps. They must apply the spirit of progressiveness to new and unexplored areas, often beyond the comprehension of their predecessors.

Research has its own exacting principles. These revolve around the choice of a real worthwhile problem, the proper methods to be used in solving the problem, the choice of the proper techniques for acquiring the information needed for solving the problem, relentless probing of the new information to see if it can be disproved or disqualified as contributing to the knowledge desired, and, finally, the use of every known device to fortify sound logic and judgment. Research is nothing more than an intelligent and proved method of solving problems. There is nothing easy or simple about solving real problems on the frontiers of science. It requires tough mental discipline, precision in experimentation, and detached, unemotional appraisal of all new facts.

These awe-inspiring demands will never be met unless the student is taught the value of fundamental principles in solving every research problem. He must project his ideas forward with precision, and this can be done only by sound, deductive reasoning from basic principles. There are two ways to start any experimentation: blind, empirical probing or reasoned advance on the most probable course. Failures are the natural product of empiricism, but even

worse is the fact that each failure teaches the investigator absolutely nothing except that this particular experiment should not be repeated. The reasoned approach from principle has a much better chance of scoring success, but even if it fails it serves to reorient one's ideas and direct the effort into an alternate channel where some other hypothesis will be applicable.

Plant pathologists probably should master more techniques than any other professional group of scientists because the subject touches upon so many fields—bacteriology, soil microbiology, chemistry, genetics, etc. The student must be taught skill in designing experiments and in carrying out precise measurements and use of instruments. It is equally important, however, that he be taught that techniques are not an end in themselves but are merely the tools required in solving problems. The technique he knows best may not be needed on a certain problem and must be ruthlessly ignored until the occasion for its use arises elsewhere.

Every generation of plant pathologists must locate its own frontiers. The only thing we can be certain of is that the frontier will change as long as the profession is progressive. Today there is a crying need for understanding the nature of disease processes. The basic chemistry and physics of pathogenesis must be worked out as quickly as possible on the major diseases. When a pathogen and its suscept enter into such a relationship as to create disease, we have much more than an abnormal plant. It is a new state of physiology, usually arising from symbiosis. In its mysteries lies a great challenge for scientists in the decades ahead.

As this challenge is met, plant pathologists will become more proficient in problem solving, and hence will become more effective in developing new methods of controlling pathogens or escaping their depredations. The neophyte plant pathologist should be trained to search for these principles. Never was there a deeper challenge or a greater opportunity to serve effectively.

RHIZOCTONIA DISEASE OF BEAN AS AFFECTED BY DECOMPOSING GREEN PLANT MATERIALS AND ASSOCIATED MICROFLORAS

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SUMMARY

Five green plant materials (amendments) were added singly to sandy soil and to Elsinboro sandy loam each naturally infested with *Rhizoctonia solani*. Initial fallow periods of 0–10 weeks were followed by plantings of susceptible Topcrop snap beans. All green amendments substantially increased the total numbers of soil and rhizosphere fungi and streptomycetes, as well as the total numbers of soil bacteria, and suppressed the Rhizoctonia disease of beans. The effectiveness of amendments was greatest when beans were planted 3-7 weeks after amendment incorporation. Decomposing green corn and oats stimulated the highest numbers of streptomycetes antagonistic to *R. solani*, gave the susceptible host the best and longest protection, and decreased the competitive saprophytic activity of *R. solani* in soil. The effectiveness of corn and oats lasted longer than that of other amendments. Sudan grass and no amendment (control) resulted in fewer antagonistic streptomycetes than corn and oats, in the highest infection indices in all plantings, and in the highest competitive saprophytic colonization by the pathogen of mature buckwheat stem pieces buried in soil. Green snap bean and buckwheat amendments were intermediate in these respects. In laboratory studies the amendments retained their relative position in reducing competitive saprophytic colonization of the substrate in the absence of the host. No relation was found between disease severity and bacterial numbers in the bean rhizosphere or between disease severity and the rhizosphere/soil ratios of bacteria, streptomycetes, or fungi.

Evidence is now abundant that *Rhizoctonia solani* Kühn can be effectively suppressed by adding decomposable organic amendments to the soil (1, 4, 10, 13, 15, 19, 20). Assumed to play an important role in this suppression are saprophytic microfloras associated with the decomposition of soil amendments (1, 23, 24).

Dry and green plant amendments applied to soil naturally infested with *R. solani* imparted to susceptible snap beans some protection from the pathogen, but the protection varied with time after incorporation (4). Severity of the Rhizoctonia disease was reduced by amendments that decreased the bacteria/streptomycetes ratios as well as increased the absolute numbers of fungi and streptomycetes in the soil and within the bean rhizosphere. The large number of dry and green amendments used in that study prevented critical assessment of factors that may influence disease severity as well as the saprophytic survival of *R. solani* in soil. The present investigation was undertaken to determine any relation between the relative abundance of certain groups of microorganisms associated with decomposing green amendments and virulence of the pathogen or its potentialities for saprophytic colonization and survival in soil. The results have been published as an abstract (17).

Materials and methods.—Continuous cropping with susceptible Topcrop snap beans so increased the inoculum of *R. solani* in a sandy soil and in Elsinboro sandy loam that 90–95% of the seedlings grown therein became heavily infected. The soils were passed through a 5-mm sieve, mixed thoroughly, and weighed into portions to receive treatment. Five green organic

amendments were added singly to these soils at the rate of 1% of the oven-dry weight of the soil and the amendment. The soil-amendment combinations were placed in No. 10 cans provided with holes for aeration and drainage. The amendments used and their stage of growth when added to the soil were buckwheat in blossom, corn in early joint, oats in early head, snap beans in blossom, and sudan grass in joint. The equivalent of about 2.5 kg of dry soil was placed in each can. Amended and unamended soils were maintained at 50% of moisture-holding capacity (MHC) by daily weighing and watering.

All treatments in the greenhouse were set up in quintuplicate. Treatments in the laboratory were set up in quadruplicate, and randomized block design was used throughout. To determine the relation of decomposition stage of the amendments to suppression of *R. solani*, Topcrop snap beans were planted 0, 1, 2, 3, 4, 5, 7, 8, and 10 weeks after incorporation.

After a 3-week growing period the seedlings were carefully removed from the soil, examined for disease symptoms, and rated on a scale of 6 degrees of infection (0–5) according to the extent of damage caused by *R. solani* (4). The pH of amended and unamended soils was determined electrometrically during each harvest. Soil and rhizosphere bacteria and streptomycetes were enumerated on dilution plates of egg albumen agar containing 50 ppm cycloheximide to suppress growth of fungi. Fungi from the same habitat were enumerated and identified on V-8 juice–dextrose–yeast-extract agar supplemented with antimicrobial agents (18). The numbers of streptomycetes from soil and the rhizosphere of bean seedlings antagonistic to *R. solani* in culture were determined by Herr's method (9).

In these studies rhizosphere samples included root tissue and adhering soil after superfluous soil had been removed by gentle tapping. Non-rhizosphere soil samples were taken at least 60 mm from the nearest roots.

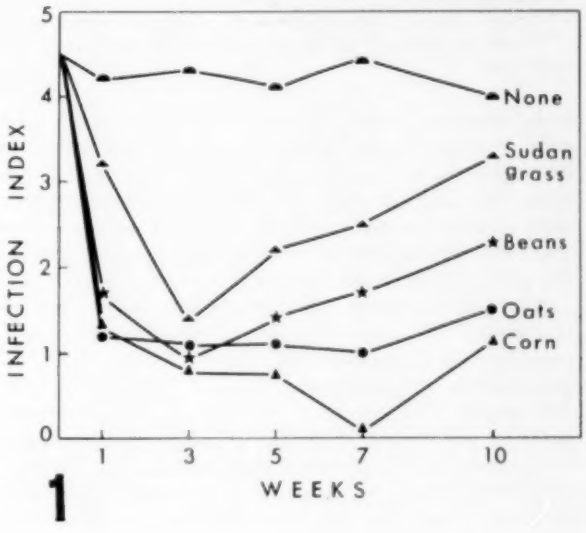
Competitive saprophytic activity of *R. solani* was determined by incubating mature buckwheat stem pieces in the soil for 4 days, during which time competitive saprophytic colonization of the pieces occurred. Results were recorded as percentage of pieces yielding *R. solani* on a suitable agar medium (16).

Experimental results.—An over-all range of soil pH of 5.9–6.8 was recorded in all experiments. The pH of amended soils did not differ appreciably from that of the controls. In no instance was soil reaction related to disease severity.

Isolations from infected bean hypocotyls yielded pure cultures of *R. solani*. Typical Rhizoctonia symptoms were produced when beans were planted in sterilized soil infested with these isolates.

Effect of decomposing green amendments on soil microflora and disease severity.—Certain green amendments added to Rhizoctonia-infested soils reduced infection considerably. The results with sandy soil (Fig. 1) indicated a general decline in infection indices. This trend lasted 7 weeks in soils amended with buckwheat (not included in Fig. 1), corn, and oats, and 3 weeks in soils amended with snap beans and sudan grass. The unamended soil did not change appreciably. For instance, beans planted 3 weeks after incorporation of snap bean or sudan grass amendments had lower infection indices than beans planted after longer intervals. Control with buckwheat, corn, and oats was best when beans were planted 1–7 weeks after incorporation. The duration of effectiveness in disease suppression seemed to depend on the rate of amendment decomposition, which depends on the nature and age of plant material and on soil conditions during decomposition (21).

Fig. 2 shows representative data for total soil bac-



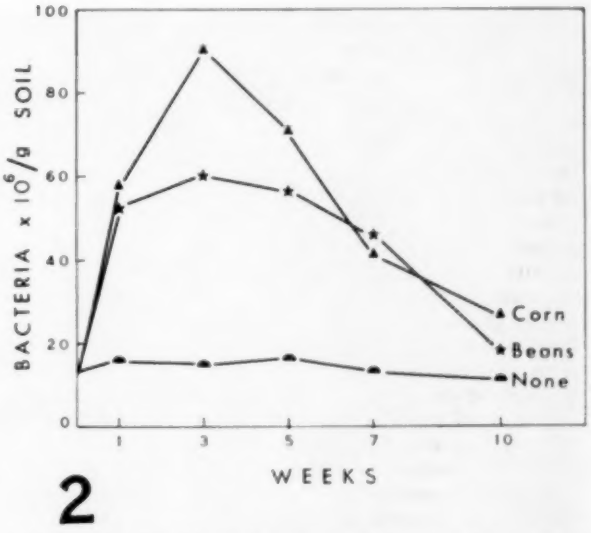


Fig. 1–2.—**1)** Effect of green amendments and time after their addition to sandy soil naturally infested with *Rhizoctonia solani* on infection indices of 3-week-old bean seedlings. **2)** Numbers of soil bacteria associated with green amendments and time after their addition to sandy soil naturally infested with the pathogen.

Table 1.—Infection indices[a] and numbers of microorganisms[b] in soil and in the rhizosphere of Topcrop snap bean seedlings as affected by green amendments added 4 weeks before planting to sandy soil naturally infested with *Rhizoctonia solani*.

Green amendment	Mean infection index	Fungi		Streptomycetes			
				Rhizosphere		Soil	
		Rhizosphere	Soil	Total	Antagonists[c]	Total	Antagonists[c]
None (control)	4.7 a[d]	798	111	7,400	720	3,000	198
Sudan grass	2.9 b	1,128	254	11,200	840	5,000	420
Buckwheat	1.6 c	913	159	16,600	760	3,650	312
Snap beans	1.5 c	1,144	122	19,400	2,140	4,250	200
Corn	0.9 cd	1,176	179	12,600	4,100	5,350	513
Oats	0.7 d	1,065	259	21,400	4,441	9,200	864

[a] Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).
[b] Thousands per g of oven-dry soil.
[c] Streptomycetes antagonistic to *R. solani* in culture as determined by Herr's method (9).
[d] Numbers followed by the same letter are not significantly different at the 5% level of probability by Duncan's multiple range test (6).

teria associated with decomposing green beans and corn. Buckwheat and sudan grass gave results similar to those from beans; oats gave results similar to those from corn. Trends with soil fungi and streptomycetes were similar to those with bacteria. It appears that the reduction in disease severity was greatest during the period of greatest microbial activity (Fig. 1, 2).

A triple-agar-layer plate technique was used to enumerate soil and rhizosphere streptomycetes antagonistic to *R. solani* (9). Fig. 3 shows plates of antagonistic streptomycetes. Fig. 4 gives representative results of these tests. Results from the 4-week planting are shown in Table 1, which also includes total numbers of rhizosphere and soil fungi and streptomycetes. Plates of antagonistic streptomycetes were prepared from the dilutions used for bacterial and streptomycetes counts.

Corn and oat amendments greatly stimulated soil and rhizosphere streptomycetes antagonistic to *R. solani* in culture and reduced disease severity of bean seedlings. Buckwheat and snap bean amendments were less effective in these respects, and sudan grass and no amendment (control) least effective. Results were similar but less pronounced in Elsinboro sandy loam naturally infested with the pathogen.

The numbers of antagonistic streptomycetes in soil amended with beans and sudan grass followed an upward trend through the fifth week, and then fell off rapidly as infection indices rose. In soil amended with corn and oats, however, the numbers of antagonistic streptomycetes remained high until the seventh week and then declined as the infection indices of bean seedlings rose (Fig. 1, 4).

Table 1 shows that all treatments made 4 weeks

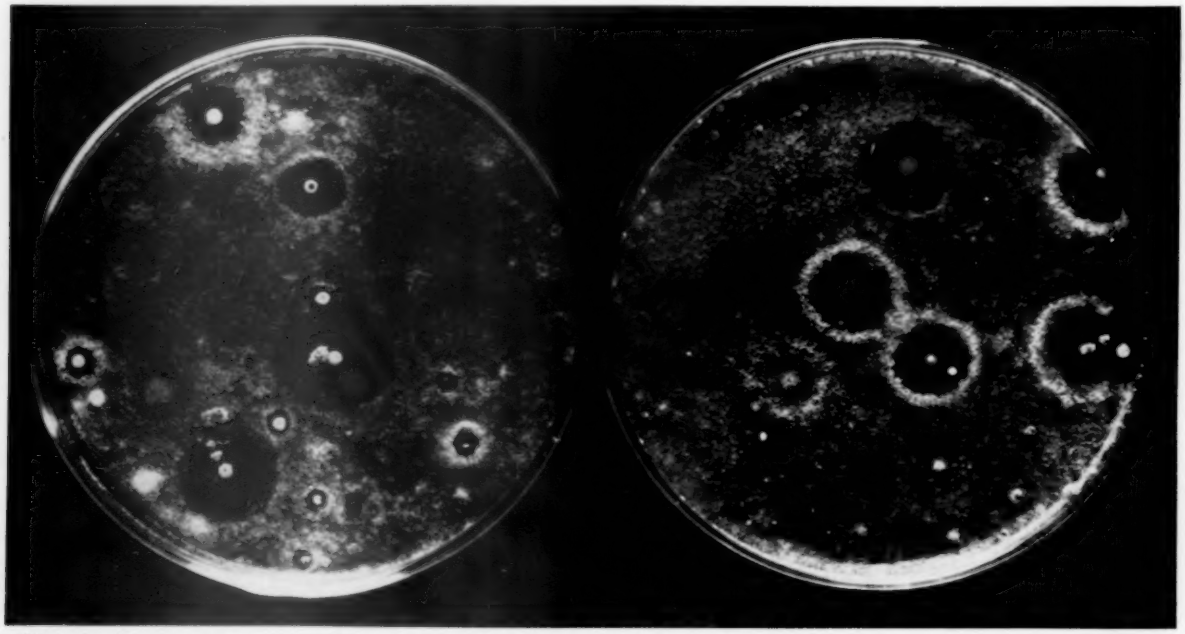


Fig. 3.—Representative assay plates of streptomycetes antagonistic to *Rhizoctonia solani*.

before planting substantially increased the total numbers of soil and rhizosphere fungi and streptomycetes and reduced the severity of infection of 3-week-old bean seedlings. With a few exceptions, the amendments associated with the greatest numbers of fungi and streptomycetes in the soil and bean rhizosphere also had the lowest infection indices. No relation was found between disease severity and rhizosphere/soil ratios of bacteria, streptomycetes, or fungi. Qualitative studies of fungal genera in soil and in the rhizosphere of beans showed no apparent relation between disease severity and total counts within the various genera.

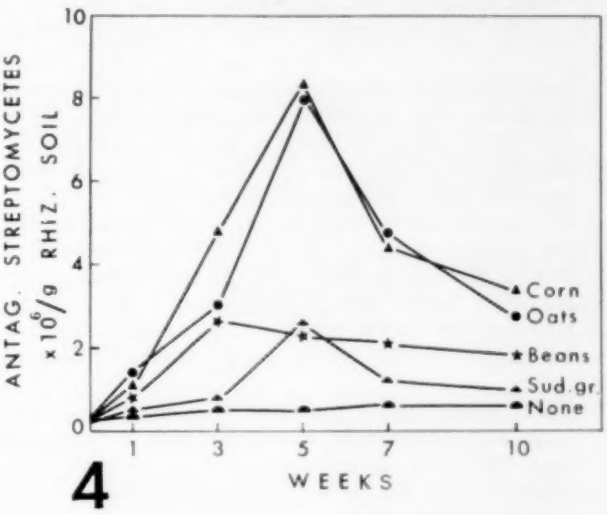
The mean infection indices of bean seedlings from the planting 4 weeks after incorporation of the amendments (Table 1) show that there were 4 levels of significance by Duncan's multiple range test (6). Oats produced significantly less disease than other treatments except corn. The effectiveness of corn and oats in controlling other plant root diseases has been observed before (5, 12, 14). All amendments produced significantly less disease than the unamended controls. Infection indices of beans in soil amended with buckwheat, corn, or snap beans were not significantly different from each other at the 5% level of probability.

Disease severity and competitive saprophytic colonization.—The relation between disease severity and the ability of the pathogen to colonize mature buckwheat stem pieces saprophytically under the influence of decomposing green amendments was determined after removal of the bean seedlings from the containers and thorough mixing of the soil. Soil and rhizosphere samples taken at the end of the experiment were analyzed for streptomycetes antagonistic to *R. solani* in culture. Representative data summarized in Fig. 5 suggested that an over-all increase in antagonistic streptomycetes in the soil and rhizosphere of seedlings may play an important role in reducing competitive saprophytic activity of the root pathogen. Sensitivity of *R. solani* to competition with other soil microorganisms upon addition of fresh organic materials to infested soil was also observed by Blair (1). Partial failure of *R. solani* to colonize suitable agar substrata through small openings of soil microbiological sampling tubes in the soil was also shown with green corn as an amendment (14). The possibility exists that an actual reduction in the total amount of inoculum per unit of soil may occur as a result of antagonistic action or some other mechanism. Competitive saprophytic colonization, however, may not indicate the total amount of inoculum in the soil at any one time under various conditions. All methods so far described fail to provide a reliable assay for both active and dormant Rhizoctonia propagules in the soil.

Decomposing amendments may produce plants less susceptible to attack through increased availability of essential nutrients. The results in Table 2 show, however, that the amendments that decreased disease severity were also responsible for the failure of the pathogen to compete successfully with other microorganisms in colonizing buckwheat stem pieces buried in soil.

Competitive saprophytic colonization in the absence of the host.—Table 3 shows the effect of green amendments on the competitive saprophytic colonization of mature buckwheat stem pieces by the pathogen in the absence of the host in the greenhouse. Colonization in sandy soil and in Elsinboro sandy loam on the day of incorporation of the amendments was respectively 75 and 51%. In the 2 unamended soils and in the soils amended with green sudan grass the competitive saprophytic activity of *R. solani* remained high throughout the experiment. The decomposition of corn in both soils appreciably reduced the competitive saprophytic activity of the pathogen. Beans, buckwheat, and oats had the same effect in Elsinboro sandy loam only.

To determine the effect of decomposing green amend-



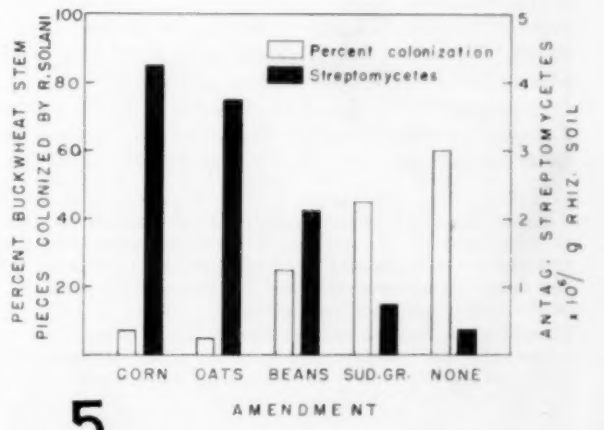


Fig. 4–5.—**4)** Effect of green amendments and time after their addition to sandy soil naturally infested with *Rhizoctonia solani* on the numbers of streptomycetes antagonistic to the pathogen in culture. **5)** Competitive saprophytic colonization by *Rhizoctonia solani* of buckwheat stem pieces buried in amended and unamended sandy soil, and rhizosphere antagonistic streptomycetes enumerated 8 weeks after addition of amendments to sandy soil naturally infested with the pathogen.

Table 2.—Infection indices[a] of Topcrop snap bean seedlings and competitive saprophytic colonization[b] by *Rhizoctonia solani* as influenced by green amendments added to sandy soil naturally infested with the pathogen.

Green amendment	9 weeks[c]		11 weeks[c]		13 weeks[c]	
	Mean infection index	Saprophytic colonization (%)	Mean infection index	Saprophytic colonization (%)	Mean infection index	Saprophytic colonization (%)
None (Control)	4.7	60	4.0	59	3.4	51
Sudan grass	2.7	46	3.2	44	3.4	51
Buckwheat	1.7	16	2.6	14	2.1	11
Snap beans	1.9	16	2.4	23	2.0	12
Oats	1.9	24	1.9	4	1.5	17
Corn	1.0	7	1.0	7	1.4	18
LSD 5%	1.2	16	1.4	13	1.7	11
LSD 1%	1.7	21	1.8	17	2.4	15

[a] Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).
[b] Per cent of buried mature buckwheat stem pieces colonized.
[c] Time from amendment incorporation in the soil to removal of 3-week-old seedlings.

ments on the competitive saprophytic colonization of *R. solani* in the absence of a susceptible host under controlled conditions in the laboratory, naturally infested unsterilized sandy soil was amended with chopped green plants including their roots, mixed thoroughly, and incubated at 25°C for 20 weeks. The soil moisture content was brought to and maintained at 60% MHC by frequent weighing and watering with tap water of the same temperature. Results were inconclusive in weekly determinations of streptomycetes antagonistic to *R. solani* in culture during the experiment.

The ability of the pathogen to colonize buckwheat stem pieces buried in unamended soil 4 days was high (56%) at the beginning of the experiment and remained high during the first 4 weeks of incubation (Table 4). In soil amended with sudan grass and snap beans the percentage of colonization dropped during the first week though the pathogen was still recoverable 20 weeks after incorporation of the amendments. Decomposition of green buckwheat, corn, and oats resulted in complete failure of *R. solani* to colonize the substrate. This failure became evident within 2 weeks of incorporation. These results agree with those of Sanford (20), who found that *R. solani* disappeared from natural soil within 17 weeks in the absence of a susceptible host (potatoes) when associated with barley, corn, oats, or wheat replanted every 3 weeks, but persisted 24 weeks or longer in soils planted with beans, peas, or potatoes.

Discussion.—The results lead to the conclusion that the degree of control obtained depends on when the susceptible host is planted after amendment incorporation. Easily decomposable plant materials gave best control when plantings were made 1–3 weeks after incorporation, whereas protection imparted by amendments more resistant to decomposition occurred from the third to the seventh week. Since reduction of the disease was most substantial in plantings made during or shortly after the time at which maximum numbers of associated microorganisms were counted, the rate of decrease in disease severity appears inversely related to the activity of soil microflora associated with the decomposing green amendments. The significance of proper timing of plantings after incorporation of amendments has also been emphasized in other experiments with *R. solani* (4, 15), and in the case of the Verticillium wilt fungus (22).

The results strongly suggest that the disease suppression is a microbiological process, but the exact mechanism of control is a matter of conjecture. It is obvious (Fig. 4, Table 1) that microbial antagonism

Table 3.—Competitive saprophytic colonization by *Rhizoctonia solani* in the absence of a susceptible host in the greenhouse as influenced by green amendments added to greenhouse loamy sand (G) and Elsinboro sandy loam (E) naturally infested with the pathogen.

Green amendment	Saprophytic colonization[a] at various intervals after amendment					
	9 weeks		11 weeks		13 weeks	
	G	E	G	E	G	E
None (control)	62	47	76	37	60	29
Sudan grass	51	44	57	45	72	31
Buckwheat	40	0	47	0	12	2
Snap beans	54	3	52	2	33	1
Oats	21	2	23	2	8	5
Corn	7	4	4	0	2	2

[a] Per cent of buried mature buckwheat stem pieces colonized.

Table 4.—Competitive saprophytic colonization by *Rhizoctonia solani* in the absence of a susceptible host in the laboratory as influenced by green amendments added to sandy soil naturally infested with the pathogen.

Green amendment	Saprophytic colonization[a] at various intervals (weeks) after amendment				
	0	1	2	4	20
None (control)	56	49	42	43	16
Sudan grass	56	18	10	8	4
Snap beans	56	14	11	5	5
Buckwheat	56	3	0	0	0
Oats	56	20	1	0	0
Corn	56	42	0	1	0

[a] Per cent of buried mature buckwheat stem pieces colonized.

may play an important role in decreasing disease severity and in suppressing the pathogen in the soil. In several instances soil amended with corn and oats had 3–4 times as many streptomycetes antagonistic to *R. solani* as unamended soil. In the rhizosphere of such cultures the difference was as great as 6-fold. The relation between disease severity and numbers of antagonistic streptomycetes, though based on *in vitro* enumeration, suggests a mechanism of control without precluding other possibilities. A reliable method to count antagonists *in situ* has yet to be devised. The importance of antagonistic microorganisms in reducing disease severity has been summarized in connection with plant root diseases (24).

Not only disease severity but the competitive saprophytic activity of *R. solani* is suppressed during the decomposition of some amendments in natural soils infested with the pathogen (Table 3, 4). Suppression of saprophytic activity may be partly accounted for by direct antagonistic action (Fig. 5). Immediately following the removal of the host, amendments that reduced disease severity appeared to be responsible for suppressing the saprophytic activity of the pathogen. Whether this suppression is an actual reduction of inoculum in the soil or a simple inactivation of inoculum cannot be concluded. Boosalis and Scharen (2) recently concluded that methods for reducing *R. solani* in soils are those that are deleterious to the sclerotia harbored on the surface of plant debris particles and hyphae within dead plant tissue. Propagules well protected in such habitats may not be permanently affected by decomposing amendments and their associated microfloras. Only a successful quantitative assay of active and dormant inoculum in the soil would provide a clear picture of the effect of various treatments on the actual amount of inoculum of *R. solani*.

If the saprophytic ability of *R. solani* is needed for survival in the soil in the presence of competing microflora associated with decomposing organic materials, evidence obtained in the absence of a susceptible host (Table 3, 4) can be interpreted to indicate that the strains present in the soil in this investigation do not depend entirely upon parasitic nutrition for persistence in soil for at least 20 weeks. Evidently, other soil factors and treatments applied may exercise a pronounced effect on the saprophytic survival of *R. solani* in soil. For instance, the pathogen was more sensitive to amendments of corn, oats, and buckwheat than of snap, beans, sudan grass, and unamended soil. Further, suppression was more pronounced in Elsinboro sandy loam than in sandy soil.

Although the effectiveness of amendments in suppressing competitive saprophytic colonization was the same in controlled laboratory experiments as in the greenhouse experiments, competitive saprophytic colonization was not found to be related to numbers of antagonistic streptomycetes. A lack of aeration in beakers maintained at 60% MHC inside closed incubators at 25°C encouraged multiplication of certain bacteria. In many instances, plates were discarded because of bacterial interference with the development of antagonistic zones.

Under the conditions of the experiments, the pathogen appears to be able to thrive in some cases despite the coincident increase of microflora associated with decomposing plant materials, but not in others. These results support Blair's (1) findings and the views expressed by Garrett (8), but they do not support Boyle's report (3) that after addition of crop refuse the inoculum of an unidentified species of Rhizoctonia was very effective in causing root rot of peanuts. Differences in various factors and the wide variation within the pathogen (7, 11) may account for the differences.—Plant Industry Station, Beltsville, Maryland.

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EFFECT OF DRY MATURE PLANT MATERIALS AND NITROGEN ON RHIZOCTONIA SOLANI IN SOIL

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SUMMARY

In a factorial experiment in the greenhouse 4 dry mature plant materials (amendments) and 2 forms of supplemental nitrogen were incorporated in Elsinboro sandy loam naturally infested with *Rhizoctonia solani*. The amendments were added at the rate of 1% of the oven-dry weight of the soil and the amendment. Soluble NH_4NO_3 and slowly available Uramite were added to provide 100 ppm of N in the soil. After an initial fallow period of 4 weeks, susceptible Topcrop snap beans were planted. Soybean, corn, and oat amendments were effective in reducing the Rhizoctonia disease of bean as well as the competitive saprophytic activity of the pathogen. Oak sawdust was inferior in both of these respects. Both nitrogen forms somewhat increased the effectiveness of all amendments but soybeans. They were also effective in reducing the disease when applied alone. The amendments and nitrogen sources were more effective in reducing the competitive saprophytic activity of the fungus in the absence than in the presence of a host. The suppression of *R. solani* in soil by mature organic amendments and supplementary nitrogen appears to be related to the general microbial activity in the soil, partially related to the C/N ratio of the amendment, and unrelated to soil reaction.

Screening tests of a large number of organic soil amendments recently showed that the effectiveness of any given amendment in suppressing *Rhizoctonia solani* Kühn in soil depended on interval after incorporation (7). Some of the mature amendments resulted in nitrogen-deficient plants. Chlorotic snap bean plants were less diseased, but were also less desirable from a practical point of view.

Several workers failed to agree on the effects of additional soil nitrogen on diseases caused by *R. solani*. Supplemental soil-nitrogen increased disease development on such suscepts as snap beans (1), bent grass (5), and lettuce (6), whereas added nitrogen decreased disease on potatoes (16) and sugar beets (18). Nitrogen was effective on sugar beets when used after winter fallow but not after winter cover crops (18). In the absence of any host the effectiveness of organic amendments in reducing growth of *R. solani* in soil was found to be impaired by the addition of nitrogen (3). Nitrogen alone, however, did not increase the growth of *R. solani* in soil.

The work reported here was undertaken to assess the value of a limited number of dry mature amendments alone and in combination with additional nitrogen in controlling *R. solani* in naturally infested soil in the presence and the absence of susceptible Topcrop snap beans. Chosen as nitrogen sources because of their differing availability characteristics were soluble NH_4NO_3 and slowly available Uramite (a urea-formaldehyde product provided through the courtesy of E. I. du Pont de Nemours and Company, Wilmington, Del.).

Materials and methods.—Elsinboro sandy loam was continuously cropped to Topcrop snap beans until 90–95% of the seedlings were heavily infected with *R. solani*. The soil was then passed through a 5-mm sieve, mixed thoroughly, weighed, and placed in tared 6-in. pots. In a factorial experiment, 4 mature amendments were added singly to the soil at the rate of 1% of the oven-dry weight of the soil and the amendment. One-third of the amendment portions were mixed with a solution of NH_4NO_3 to give 100 ppm of nitrogen in the soil. Another third received Uramite at the same rate of nitrogen application, and the final third received no additional nitrogen. The unamended soil portions also received the same nitrogen treatments. The amendments were mature corn stover, oat straw, soybean hay, and oak sawdust. The treatments were set up in quintuplicate in a randomized block design in the greenhouse, and the soil was maintained at 50% of the moisture-holding capacity (MHC) throughout the experiments by daily weighing and watering. Since other work (7) had shown that most amendments were effective 3–7 weeks after incorporation, a 4-week fallow period was allowed before the snap bean host was planted.

After a 3-week growing period the seedlings were carefully removed from the soil, and rated for disease development, and samples were taken for the enumeration of soil and rhizosphere microflora. The methods and materials have been described (7, 15). The competitive saprophytic activity (10) of *R. solani* was determined by a colonization method (14). The pH of all soils was measured electrometrically at time of harvest.

Table 1.—Effect of mature organic amendments and 2 nitrogen forms on the infection indices of beans in soil naturally infested with *Rhizoctonia solani*.

Amendment	Infection index[a] with stated nitrogen source			
	None	NH_4NO_3	Uramite	Mean
Soybeans	0.06	0.58	0.53	0.39
Corn	0.62	0.34	0.53	0.50
Oats	0.71	0.79	0.33	0.61
Sawdust	2.88	1.84	1.79	2.17
None (control)	2.81	1.89	2.23	2.31
Mean	2.36	1.81	1.80	

[a] Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).

The same soil was used in the laboratory to study the activity of *R. solani* in the absence of a susceptible host. The amendments and nitrogen sources were applied separately to evaluate their ability to suppress the pathogen. The nitrogen was applied to the soil at both 100 and 200 ppm. The treated soils were placed in 600-ml beakers maintained at 50% MHC and incubated at 25°C for various periods. The status of *R. solani* was determined at various desired times by selecting 4 replications of each treatment at random and conducting the competitive saprophytic colonization test. These studies have been abstracted (8).

Experimental results.—*Effect of amendments and nitrogen on soil reaction.*—An over-all soil pH range of 5.5–6.6 was recorded in all experiments. Soil acidity increased about 0.3 pH units from nitrification of the ammoniacal nitrogen from the NH_4NO_3, and, to a small degree (about 0.1 pH unit) from the slowly available Uramite. All 4 amendments decreased soil acidity somewhat, but in no instance could changes in disease severity be attributed to changes in soil reaction.

Effect of amendments and nitrogen on soil and rhizosphere microflora and disease severity.—Except for sawdust that had not received supplemental nitrogen, all 4 amendments reduced infection indices below those of the controls (Table 1). Ability to suppress the disease, however, differed substantially. Soybeans were particularly effective, and followed closely by corn and oats. Sawdust was either ineffectual or increased the disease slightly. The 2 nitrogen sources were about equally effective in reducing disease severity. Their apparent value, however, varied with the amendment, and their effectiveness was much less pronounced than that of soybeans, corn, or oats. When no organic amendment was used, soluble NH_4NO_3 reduced Rhizoctonia disease of bean more than the slowly available Uramite.

The soil and rhizosphere microflora did not respond appreciably to supplemental nitrogen. The limiting factor for microbial growth in the unamended soil was apparently an inadequate organic energy source rather than a shortage of nitrogen. The data presented (Table 2), therefore, are the mean effects of the amendments irrespective of the added nitrogen. In the soil, all amendments were responsible for increases in numbers of fungi, streptomycetes, and bacteria. Soybeans and oats appeared to stimulate soil fungi and bacteria, and corn stimulated soil streptomycetes. In the rhizosphere corn, oats, and soybeans stimulated bacteria and streptomycetes. Sawdust had a microbial-suppressing effect on fungi and bacteria in the rhizosphere.

Table 2.—Mean effect of mature organic amendments on numbers[a] of soil (S) and bean rhizosphere (R) microorganisms.

Amendment	Fungi		Streptomycetes		Bacteria	
	S	R	S	R	S	R
	$\times 10^3$	$\times 10^6$	$\times 10^6$	$\times 10^6$	$\times 10^6$	$\times 10^6$
Soybeans	369	2.6	6.4	17	60	290
Corn	221	2.6	7.3	19	44	337
Oats	317	3.3	6.3	18	61	262
Sawdust	289	1.5	6.6	12	45	217
None (control)	195	2.6	3.7	11	31	242

[a] Numbers of microorganisms per g of oven-dry sample.

Effect of amendments and nitrogen on competitive saprophytic colonization in the presence of a host.—Immediately after the bean seedlings were removed, and the soil thoroughly mixed, the competitive saprophytic colonizing activity of *R. solani* was determined by the buckwheat method (14). Colonization results (Table 3) agreed with the infection indices (Table 1). Soybeans, corn, and oats appeared to be superior to sawdust in suppressing the competitive saprophytic activity of *R. solani* in soil. In general, both forms of nitrogen reduced the competitive saprophytic activity of *R. solani*. The soluble NH_4NO_3 was equal to or better than the slowly available Uramite.

The close parallel between the degree of competitive saprophytic colonization of the buckwheat stem pieces by *R. solani* and the infection indices (Tables 1 and 3) confirmed the usefulness of the colonization method in assaying active *R. solani* in the absence of a host.

Effect of amendments, nitrogen, and time on competitive saprophytic colonization in the absence of a host.—In order to understand more clearly the direct action of both the amendments and nitrogen sources on *R. solani*, portions of the soil were taken to the laboratory, where temperature and moisture could be

Table 3.—Effect of mature organic amendments and 2 nitrogen forms on the competitive saprophytic colonization by *Rhizoctonia solani* in soil in the presence of a susceptible host.

Amendment	Colonization[a] with stated nitrogen source			
	None	NH_4NO_3	Uramite	Mean
Soybeans	4	1	4	3
Corn	11	9	9	10
Oats	12	8	11	12
Sawdust	35	16	16	22
None (control)	36	27	35	32
Mean	32	20	25	

[a] Per cent of buried mature buckwheat stem pieces colonized.

Table 4.—Effect of mature organic amendments and time after their incorporation in soil on the competitive saprophytic colonization by *Rhizoctonia solani* in the absence of a susceptible host.

Amendment	Colonization[a] at various intervals (weeks) after addition of amendments				
	0	2	4	6	8
Soybeans	42	0	1	0	1
Corn	42	4	6	6	2
Oats	42	2	4	1	1
Sawdust	42	20	38	35	16
None (control)	42	25	46	33	28

[a] Per cent of buried mature buckwheat stem pieces colonized.

more accurately controlled. The amendments and nitrogen were applied to separate lots of soil at the same rates as in the greenhouse since the greenhouse experiments had not disclosed any striking synergistic action by them on *R. solani*. The competitive saprophytic colonization tests were conducted at 2-week intervals, up to 8 weeks. The results (Table 4) indicated that the soybean, corn, and oat amendments were more effective in suppressing *R. solani* in soil in the absence of the host than when the host was present. Soybeans substantially reduced the competitive saprophytic colonizing activity of *R. solani* for at least 8 weeks. The percentage of buckwheat pieces colonized was always greater in the sawdust-treated soil and the unamended controls than in the soil containing the other amendments.

In the test of the nitrogen sources, the high rate of application (200 ppm) of both forms almost maintained the initial colonizing activity of the pathogen during the first week (Table 5). At the end of the second week, however, both nitrogen sources produced some suppression of *R. solani*. Comparing these results with those in Table 4 it appears that the nitrogen sources employed were much less effective than the amendments soybeans, corn, and oats. The low initial colonization in the nitrogen test (30%), compared with 42% in the amendment test, may be accounted for by the fact that the soil portion reserved for the nitrogen test was stored 8 weeks at 5°C before being used.

Discussion.—Differences between the 4 dry mature amendments in reducing the Rhizoctonia disease of bean and the competitive saprophytic activity of *R. solani* in soil may be partially accounted for by their relative rates of decomposition. Corn, oats, and soybeans decomposed more rapidly than oak sawdust, and controlled *R. solani* in soil more effectively. In the absence of an organic amendment the nitrogen sources also reduced the parasitic and saprophytic activities of *R. solani* in soil. Soluble NH_4NO_3 was more effective than slowly available Uramite. When Das and Western (6) added nitrogen to soil they found an increase in the disease of lettuce caused by *R. solani*. Perhaps the influence of the kind of host on the effect of the added nutrient may explain the different effects of nitrogen on beans and lettuce.

In laboratory tests, the amendments were more effective in the absence of a susceptible host than in the presence of a host. The nitrogen sources, when added alone, reduced the competitive saprophytic activity of the pathogen after 2 weeks. Evidently the pathogen, through parasitic nutrition, partially overcame the suppressing effect of the amendments and nitrogen sources.

Soil reaction below pH 5.8 has been reported to affect free and independent growth of *R. solani* in unsterilized soil (3), but the pH range in these studies (5.5–6.6) was not a critical factor. Both high and low infection indices were recorded above and below pH 5.8. Because of nitrification, nitrogen sources slightly decreased soil pH, but soil reaction had no apparent relation to infection indices. These results are in agreement with those of previous work on the infectivity and persistence of *R. solani* in soil (7, 16, 17).

On the basis of the relative effectiveness of the 4 amendments alone it might appear that the lower the carbon/nitrogen (C/N) ratio of the amendment the greater its effectiveness in suppressing the pathogen. The relationship, even if valid, is not simple. The nitrogen-enriched sawdust carried more nitrogen per unit of carbon than did the unenriched oat straw, and yet it was not nearly as effective. In addition, many green amendments with low C/N ratios actually increased the Rhizoctonia disease of beans whereas dry amendments hardly ever increased it above that in unamended soil (7, 12, 13). Finally, the soybeans, which had the lowest initial C/N ratio of the amendments used in this study, were rendered less effective by the supplemental nitrogen (Table 1). Possibly there is a C/N ratio range within which amendments reach a maximum effectiveness and above or below which their effectiveness decreases. The answer, however, must await further studies.

The stimulation of microflora associated with the decomposition of the 3 amendments that suppressed Rhizoctonia disease suggests that this suppression is a result, at least in part, of a general increase in microbial activity in the soil. This does not rule out a major role for certain specific microorganisms. The stimulation of over-all microbial activity, however,

Table 5.—Effect of forms of nitrogen, rate of application, and time after incorporation in soil on competitive saprophytic colonization by *Rhizoctonia solani* in the absence of a susceptible host.

Nitrogen form and rate (ppm)	Colonization[a] at various intervals (weeks) after addition of nitrogen		
	0	1	2
None (control)	30	18	12
NH_4NO_3			
100	30	10	12
200	30	26	8
Uramite			
100	30	20	6
200	30	24	5

[a] Per cent of buried mature buckwheat stem pieces colonized.

would seem plausible in view of Blair's (3) findings on the CO_2 sensitivity of *R. solani* and recent work by Durbin (9), who showed that strains of the fungus attacking the host at or near the ground line are particularly sensitive to CO_2. The strains of the pathogen encountered in this study attacked the bean hypocotyls at the ground line.

It would be of particular interest to determine whether the amendments and nitrogen sources under consideration were actually responsible for a partial eradication of *R. solani* from soil or merely for a suppression of its activity. Herzog and Wartenberg (11) recently showed that sclerotia of *R. solani* that remained inactive in soil for 5 years in the presence of "antibionts" could infect a host. These "antibionts" were described as biological breakdown products of organic amendments. There is little doubt that colonization tests (2, 14) will indicate an increase in the number of propagules of the pathogen present as increasing amounts of inoculum are added to soil (up to a certain limit). It appears purely speculative, however, to assume that the converse is also true. Reduction in the colonizing activity by soil treatments does not necessarily indicate a reduction in the number of propagules of the pathogen per unit of soil. It does appear safe, however, to assume that the activity of the pathogen has been reduced. More studies are needed on the question of eradication or inactivation. Boosalis and Scharen (4) recently proposed methods that might be adapted to this problem.—Plant Industry Station, Beltsville, Maryland.

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INTERACTION OF POTASSIUM GIBBERELLATE AND A STUNTING BEAN VIRUS ON BEANS, PHASEOLUS VULGARIS

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SUMMARY

In plants of a vining bean variety (Bayo 158), 1) 50 ppm potassium gibberellate stimulated growth rate almost immediately, through internode lengthening; 2) Mexican Severe Bean Mosaic Virus slowed growth considerably, taking effect several days after inoculation and almost stopping growth in the second week after inoculation; 3) potassium gibberellate only slightly stimulated previously infected plants, and was almost completely counteracted by subsequent inoculation.

The Severe Bean Mosaic Virus (SvBMV) (5) in Mexico stunts bean plants decidedly by reducing internode lengthening. Seedling growth slows down considerably even before other symptoms, such as mottling, are appreciable. The slowing is measurable about 7 days after inoculation, and growth thereafter is very slight. Plants remain stunted permanently.

A 50-ppm solution of potassium gibberellate applied to bean seedlings markedly increases growth by elongating internodes. The response is measurable within 2 days of treatment. Growth continues accelerated for about 14 days and then returns to normal. This limited effect was demonstrated by Brian and Hemming (2). Barton (1) and Maramorosch (3) demonstrated that gibberellic acid could reverse physiologic dwarfing and

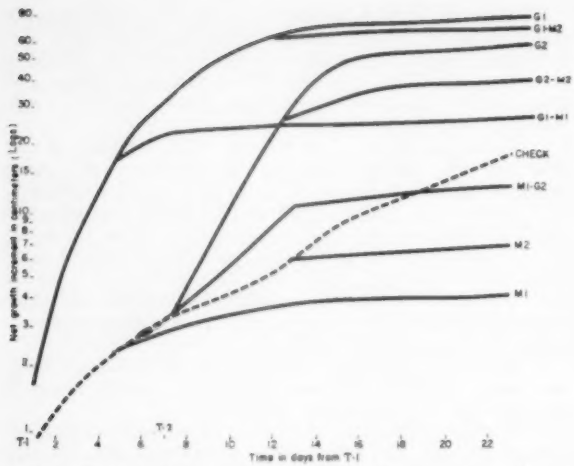

Fig. 1.—Graph of growth data for all treatments, inoculations, and combinations. G = potassium gibberellate treatment; M = mosaic inoculation; numbers following letters refer to time of treatment or inoculation. Growth increment figures are in centimeters on a logarithmic scale.

virus-caused stunting. Reported here is a study of interactions between the Mexican bean stunting virus and a gibberellic acid derivative (4).

Methods.—Plants of a vining bean variety (Bayo 158), at 2 different growth stages, were treated with a 50-ppm solution of potassium gibberellate (G), inoculated with the Mexican Severe Bean Mosaic Virus (M), or given combinations of both treatment and inoculation (MG or GM). The 2 growth stages were full expansion of primary leaves (Time 1) and 7 days later (Time 2). All treatments and inoculations were on primary leaves. Inoculations were made with carborundum and juice expressed from diseased plants; treatments were made by wiping leaves with cotton pads saturated with the potassium gibberellate solution. Plant height (distance from soil level to growing point of the main stem) of ten plants in each plot was measured daily for over 3 weeks. The data are expressed as the average daily net height increase of ten plants in each plot from Time 1. Differences between the average net plant heights were used as a measure of growth rates and total growth over any given period.

Results.—Fig. 1 graphs data for all treatments, inoculations, and combinations. Growth figures are in centimeters on a log scale.

Normal seedling growth (Check).—The growth of untreated uninoculated check plants was almost a straight log-line function. During the period studied there was no tendency toward a leveling-off of the growth rate (Fig. 1).

Growth response to potassium gibberellate (G1 and G2).—Potassium gibberellate administered at Time 1 (G1) gave an almost immediate growth response, measurable on the next day. Growth acceleration continued until the 14th day after treatment, at which time the growth rate approached that of the check plants. Growth for 4–10 days after treatment was 10–20 times as fast as that of the check plants. At the end of 3 weeks G1 plants were 4 to 5 times as tall as the check plants. G2 plants, with a slightly smaller growth rate response, were after 3 weeks about 4/5 as tall as G1 plants. Treatment at Time 2 lasted longer, the acceleration persisting to about 18 days after treatment. This phenomenon was observed with several bean varieties. The magnitude of response to potassium gibberellate appears to diminish as seedlings become older, whereas duration of response increases slightly (Fig. 1).

Response to inoculation with the Mexican bean virus (M1 and M2).—The growth rate of plants inoculated at Time 1 or Time 2 closely paralleled that of the checks until 7 days after inoculation, when it decreased measurably and steadily diminished thereafter. At the end of 12 days plant height was about 50% that of the checks. From the 13th day on, there was very slight daily growth or none at all (Fig. 1). Fig. 2 compares check, inoculated, and treated plants.

Fig. 2.—G1-treated, M1-inoculated, and check plants 13 days after Time 1.

Fig. 3.—G1-treated, M1-inoculated, and G1-M1 treated-inoculated plants 13 days after Time 1.

Fig. 4.—G2-treated, M1-inoculated, and M1-G2 inoculated-treated plants 13 days after Time 1 (6 days after Time 2).

Simultaneous potassium gibberellate treatment and virus inoculation (G1-M1 and G2-M2).—Response to G1-M1 treatment-inoculation was initially similar to that obtained from G1 treatment. After 5 days, however, growth rate decreased measurably. By the 11th day it was very small or nonexistent. The plants were taller than M1 or M2 plants, but much shorter than G1 or G2 plants (Fig. 3). Apparently the action of the virus begins to overcome the stimulus of the potassium gibberellate about 5 days after simultaneous treatment and inoculation. The fact that growth rate decreased before it did in G1-treated plants indicates that the virus was counteracting the stimulus of potassium gibberellate.

Results were similar with the G2-M2 treatment-inoculation. Growth rate was slowed by virus action about 8 days after treatment-inoculation, again earlier than in plants treated only with potassium gibberellate (Fig. 1).

Treatment with potassium gibberellate at Time 1 and inoculation with the virus at Time 2 (G1-M2).—As before the plants responded immediately to potassium gibberellate. Five days after inoculation at Time 2, growth rate slowed measurably. Ten days after inoculation, growth was very slow and plant height did not increase significantly thereafter (Fig. 1).

Inoculation with the virus at Time 1 and treatment with potassium gibberellate at Time 2 (M1-G2).—For seven days after inoculation, plant growth was similar to that of the check. After treatment with potassium gibberellate at Time 2, growth rate increased slightly, but only ⅓ to ¼ that of G2 plants. By 8 days after Time 2 growth was very slow. Potassium gibberellate had elicited a growth response before expression of virus stunting, but the virus subsequently offset the stimulus and held growth rate below that of the check (Fig. 1, Fig. 4).

Discussion.—These preliminary results point up phenomena worthy of further study. First, the apparent simultaneous activity of the virus and the growth stimulator suggests that the two factors are working at different loci within the physiologic framework of the plant. This is seen especially in the M1-G2 experiment, where plants infected for 1 week responded to potassium gibberellate, though only very slightly.

Second, stunting symptoms appeared slightly earlier in the G1-M1 and G1-M2 experiments than in the M1-G2 and G2-M2 experiments. Further study should determine whether gibberellin predisposes these very young plants to faster stunting. It may be only that the stunting is more easily measurable because of greater growth stimulus to very young plants. It is also apparent that different virus-host series vary in their response to gibberellic acid derivatives. As already mentioned, Maramorosch (3) found that gibberellic acid overcame the respective stunting effects of wound tumor, aster yellows, and corn stunt viruses on crimson clover, aster, and corn. In contrast, the Mexican Severe Bean Mosaic Virus overcame the stimulus of a gibberellic acid derivative. Certainly, the interaction between a growth stimulator and a stunting virus can be a tool for both plant-growth and plant-disease studies.—The Rockefeller Foundation, New York, N. Y.

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PHYSIOLOGIC SPECIALIZATION OF FUSICLADIUM EFFUSUM AND ITS EVALUATION IN VITRO

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SUMMARY

Cultures of *Fusicladium effusum* from the pecan varieties Squirrel, Western, and Sovereign were separated into 3 physiologic races (respectively designated races 1, 2, and 3) on the basis of pathogenicity in the greenhouse to 4 pecan varieties. Pathogenicity was similar for single-spore and field inocula from the variety Western. Squirrel and Burkett natural inocula in the field were unable to parasitize Western, constituting an additional race (race 4) of the fungus. Four isolates from the varieties Mahan, Moneymaker, Schley, and Success failed to be pathogenic on other than their original hosts in the few greenhouse cross-inoculations attempted. For all isolates, self-inoculations resulted in the highest number of normal, sporulating lesions. Various cross-inoculations produced different types of abnormal lesions and levels of sporulation on the host varieties. The germ-tube lengths of *F. effusum* conidia from 4 host varieties growing on water agar near leaflet discs of 5 varieties fell into categories predicted by the pathogenicity of these conidia in 16 of 19 cases.

The existence of at least 3 strains or races of *Fusicladium effusum* Wint. (*Cladosporium effusum* (Wint.) Demaree) (11), the incitant of the scab disease of pecans, was reported in Georgia and Florida in 1929 (7). A possible additional race of *F. effusum* capable of attacking the Stuart variety, long regarded as highly

Table 1.—Summary of reactions of 4 western pecan varieties inoculated in the greenhouse with *Fusicladium effusum* from each of the same 4 host varieties.

Source of pathogen			Disease reaction[a] of host varieties tested			
Variety	Location (Okla.)	Code	Sq	B	W	So
Squirrel[b]	Catoosa	Sq	S	S	S	S
Burkett	Stillwater	B	–	S	–	S
Western	Paden	W	R	R	S	S
Sovereign	Muskogee	So	R	R	R	S

[a] Key to disease reactions: S = susceptible; R = resistant; – = no data.

[b] Natural inoculum from nut shucks was the source of the pathogen from the Squirrel variety. One single-spore isolate was used from each of the other varieties listed.

resistant, was recently found in Mississippi and Louisiana (2, 3). The occurrence in the 1940's of scab on the Squirrel variety, once popular in the western part of the Pecan Belt because of its scab resistance and horticultural qualities (6), demonstrated a need for study of the degree of physiologic specialization of *F. effusum* in the western pecan area. Herein is presented evidence for the existence of at least 4 races of *F. effusum* in Oklahoma. During the study, inoculum from lesions on 1 variety often failed to produce normal lesions on other varieties. Various expressions of hypersensitivity were common in cross-inoculations. An effort was therefore made to measure effects of host tissue on growth of the pathogen *in vitro* and to relate the results to the pathogenicity of the races of the pathogen used. Preliminary studies in 1956 indicated that freshly cut leaflet discs of several pecan varieties placed on water agar had no influence on the germination percentage of conidia of various races seeded on the agar or on the morphology of germinating conidia, but that the length of germ tubes of the conidia appeared to be influenced differentially by the leaflet discs. Measurements of conidial growth near leaflet discs are reported herein. An abstract has appeared (5).

Materials and methods.—*Inoculation studies.*—For greenhouse inoculations, mycelial and spore suspensions of single-spore isolates of *F. effusum* isolated from the western pecan varieties Burkett, Western, and Sovereign and the eastern pecan varieties Mahan, Moneymaker, Schley, and Success were obtained from cultures growing on Chinese chestnut agar (4). Inoculum from scabbed nut shucks of the Squirrel variety was also used.

Potted pecan trees of those varieties, grown in 4-gal. crocks, were sprayed with inoculum from cultures or from scabbed nut shucks, or inoculum from cultures or shucks was allowed to drip onto foliage. Water was atomized into the air around the trees for 48 hours following inoculation. The air temperatures around the trees during this period averaged 22°C, but inoculations were successful between 19 and 27°C.

Scab occurred only at low levels at best in greenhouse tests, and, to reduce the possibility of reporting escapes as unsuccessful cross-inoculations, every cross-inoculation reported was accompanied by a parallel successful self-inoculation. Average incubation period was 21 (range 14–27) days in the greenhouse, as compared with 10 (range 6–14) days reported by Demaree and Cole (7) from the field.

Inoculations were conducted in the field in 1957 to parallel previous greenhouse tests. Scabbed nut shucks of the Squirrel, Burkett, and Western varieties were placed in small wire-mesh cages. Early in the spring 4 cages of scabby nut shucks from each of the 3 varieties were randomly suspended above scab-free unopened buds on single trees of these same 3 varieties. The number, morphology, and spore production of scab lesions were determined in June, 1957, on 12 leaves growing beneath each cage. The number of scab lesions on randomly chosen branches not near cages was also determined for each tree.

Leaflet disc studies.—Leaflet discs 10 mm in diameter were cut with a Ganong leaf punch from healthy leaves gathered from unsprayed trees in an orchard near Stillwater, Oklahoma, in July, 1957. Conidia from scabbed nuts of the Burkett, Sovereign, Squirrel, and Western varieties obtained from the same orchard were brushed into 0.1% Tween 20 in distilled water. The racial identities of inoculum from this orchard were assumed to be the same as those found elsewhere in Oklahoma on those varieties (Table 6). Petri plates of 2% water agar were seeded with the conidial suspensions. The leaflet discs were pressed, ventral surface down, onto the seeded plates, 3 discs to a plate. After incubation in darkness for 48 hours at 25°C all growth was stopped by adding 0.1% $HgCl_2$ to the plates. The germ-tube lengths of conidia within the width of 1 microscope field (200×) of the margin of the discs were measured with an eyepiece micrometer. The first 10 germ tubes encountered near each disc were measured for 3 discs on each of 2 Petri plates for each combination of host variety and conidial source. Conidia are identified according to the host from which they were obtained (e.g., Squirrel conidia). Leaflet discs of varieties other than the one from which a given lot of conidia were obtained are called "other" discs, whereas leaflet discs originating from the same variety as a given lot of conidia are called "own" discs. Germ tubes on water agar plates not containing leaflet discs were also measured for each conidial source.

Results.—*F. effusum from western pecan varieties, greenhouse.*—The disease reactions of 4 western pecan varieties in the greenhouse are reported in Table 1 as susceptible (S) or resistant (R). In every inoculation series, self-inoculation resulted in the highest lesion number. Demaree and Cole (7) had similar results with other varieties. Among the isolates from Burkett, Western, and Sovereign and the natural inoculum from Squirrel tested on these same 4 hosts there were marked differences in host range. Three separate races can be distinguished: 1) the Squirrel conidia, pathogenic to all 4 hosts; 2) the Western isolate, which did not have as wide a host range as the Squirrel conidia (and which was unable to attack the scab-susceptible eastern variety Success); and 3) the Sov-

Table 2.—Number of lesions, morphology, and sporulation of lesions on foliage of 3 pecan varieties inoculated in the field with *Fusicladium effusum*.

Source of pathogen		Pecan varieties		
Variety	Location	Squirrel	Burkett	Western
Squirrel	Paden, Okla.			
lesions/leaf		60 ± 24 [a]	46	0
lesion types [b]		N	N+Y	X
no. spores/field [c]		45	95	0
Burkett	Okemah, Okla.			
lesions/leaf		40	93 ± 41 [a]	1
lesion types		N+Y+F	N	F
no. spores/field		50	70	0
Western	Okemah, Okla.			
lesions/leaf		0	2	53 ± 30 [a]
lesion types		X	Y	N
no. spores/field		0	0	95

[a] Fiducial interval giving fiducial limits at the 99% confidence level for each inoculum source.
[b] Key to symbols for lesion types: N = normal, Y = normal-size lesion bounded by a yellow margin of chlorotic host tissue, F = brown fleck (about 0.5 mm in diameter), X = no lesion.
[c] Number of *F. effusum* conidia per 200× microscope field in conidial suspensions from leaf lesions.

ereign isolate, only pathogenic to the Sovereign variety. The racial identify of the Burkett isolate is uncertain because of lack of data.

Because the Burkett, Western, and Sovereign isolates used each came from a single spore, the possibility that mechanical mixtures of races were being tested was virtually eliminated. The Western single-spore isolate used remained constant in its pathogenicity to a given set of hosts when inoculations were made 16 months apart. All 4 inoculum sources from western varieties failed to infect the Stuart variety.

None of the leaf lesions on Burkett, Sovereign, or Western trees inoculated with Squirrel inoculum produced spores, whereas Squirrel self-inoculations sporulated abundantly. Distinctive yellow rings occurred around the lesions on Burkett leaves inoculated with Squirrel inoculum. Such pronounced hypersensitivity was not found in other cases of successful cross-inoculations in the greenhouse.

F. effusum from western pecan varieties, field.—Leaf-lesion counts, types of lesions, and levels of conidial production on the lesions in the field inoculation test are recorded in Table 2. Control leaves not near caged inoculum sources remained virtually free of lesions. Self-inoculation levels were significantly higher than cross-inoculation levels in 2 of the 3 cases and approached significance in the third. Squirrel and Burkett inoculum sources were qualitatively similar to each other in pathogenicity on the 3 host varieties. Western inoculum differed from them markedly in pathogenicity and resembled greenhouse Western inoculum, indicating the existence of at least 2 physiologic races among these 3 natural inoculum sources. The Squirrel and Burkett inoculum appear to represent an additional race less pathogenic than that from Squirrel tested in the greenhouse.

Various types of symptom expression occurred, depending on the particular host-pathogen combination (Table 2). Green leaf tissue bounded the margins of lesions resulting from self-inoculation. Symptom expression resulting from cross-inoculations was of the following 4 types (2 or even 3 of the various lesion types occurring in some cross-inoculations): a) no lesions, e.g., Squirrel inoculum on Western, and vice versa; b) brown fleck lesions about 0.5 mm in diameter, e.g., Burkett inoculum on Western, and to some extent on Squirrel; c) normal-sized lesions surrounded by a halo of chlorotic host tissue several millimeters wide, e.g., Western inoculum on Burkett, and, in part, Squirrel inoculum on Burkett, and vice versa; and d) normal-sized lesions surrounded by green host tissue, e.g., in part, Burkett inoculum on Squirrel, and vice versa.

Normal lesions produced abundant conidia. Some chlorotic lesions produced good crops of conidia, but all lesions were sterile in the case of Western lesions on Burkett, which were all yellow. A few conidia were produced on some fleck lesions.

F. effusum from eastern pecan varieties, greenhouse.—A few self- and cross-inoculations were made among the Mahan, Moneymaker, Schley, and Success varieties with single-spore cultures isolated from the same 4 varieties (Table 3). Only self-inoculations were successful, and on the basis of reciprocal inoculations the Moneymaker and Schley isolates were distinct. The

Table 3.—Summary of reactions of 4 eastern pecan varieties inoculated in the greenhouse with *Fusicladium effusum* from each of the same 4 host varieties.

Source of pathogen			Disease reaction [a] of host varieties tested			
Variety	Location	Code	M	Mm	Sc	Su
Mahan	Shreveport, La.	M	S	–	–	–
Moneymaker	Shreveport, La.	Mm	–	S	R	–
Schley	Albany, Ga.	Sc	–	R	S	–
Success	Shreveport, La.	Su	R	–	R	S

[a] Key to disease reactions: S = susceptible; R = resistant; – = no data.

Table 4.—Germ-tube lengths of *Fusicladium effusum* conidia from lesions on 4 different pecan varieties after incubation for 48 hours near leaflet discs of 5 pecan varieties.

Leaflet disc of variety:	Germ-tube lengths (μ) of conidia from lesions on			
	Squirrel	Burkett	Western	Sovereign
Squirrel	259	245	213	180
Burkett	209	226	223	216
Western	199	203	235	209
Sovereign	210	220	237	281
Stuart	190	194	208	242
No discs	256	175	280	232

Schley isolate failed to infect Squirrel and Western. The relationships of the 4 eastern isolates to the 4 races of western origin, to the 3 races recognized by Demaree and Cole (7), or to the Stuart race (2, 3) are unknown because of lack of cross-inoculation data.

Effusum germ-tube growth near pecan leaflet discs.—The germ-tube lengths of 4 sources of conidia growing near leaflet discs of 5 pecan varieties are presented in Table 4. Data from each conidial source were analyzed separately, but water agar controls, which are included in Table 4, were variable as compared to "own" discs, and were not incorporated into the analysis. Differences in germ-tube lengths between individual disc treatments were not significant. Within each conidial source statistical comparisons (12) were made between germ-tube lengths near discs of varieties known to be susceptible and those known to be resistant (Table 5). Growth near susceptible discs exceeded that near resistant discs. For Burkett conidia the comparison was significant at slightly above the 10% level. Camparisons for the other 3 conidial sources were significant at the 5 or 1% level. When all conidial sources were pooled, the mean germ-tube length was significantly shorter (1% level) for conidia near "other" discs (212 μ) than near "own" discs (250 μ).

Autoclaved decoctions of leaves of susceptible and resistant pecan varieties failed to influence radial growth of *F. effusum* isolates differentially. Other methods of concentration or purification of the substances presumably responsible for the differential leaflet disc effects were not undertaken.

Discussion.—*Physiologic races.*—Greenhouse and field cross- and self-inoculation data strongly support the conclusion of Demaree and Cole (7) that physiologic specialization exists within *F. effusum*.

There was evidence that more than 1 race occurs naturally on a given host (Squirrel variety) in the field in Oklahoma. More inoculations with single-spore isolates are needed to establish the number of different physiologic races of the pecan scab fungus that occur in Oklahoma on the same host variety.

Three distinct races of *F. effusum* can be recognized from the greenhouse studies, 1 each from Squirrel, Western, and Sovereign varieties. For reference, these races might be respectively designated 1, 2, and 3. Western inoculum used in the field was similar to race 2 as far as tested. Burkett and Squirrel inocula were similar in pathogenicity in the field, even though Burkett self-inoculation produced significantly more lesions than the comparable Squirrel cross-inoculation, and cross- but not self-inoculations with both Burkett and Squirrel conidia evoked hypersensitivity reactions in these 2 hosts. It is suggested that the Burkett and Squirrel inoculum sources listed in Table 2 be both designated race 4. Table 6 is a key to the 4 *F. effusum* races described from Oklahoma sources. The relationships of these 4 races to the other isolates described in this paper and to races described elsewhere (2, 3, 7) are unknown.

Bailey (1) noted that, as new tomato varieties resistant to the known races of *Cladosporium fulvum* Cke. were grown commercially, new races of the pathogen developed capable of attacking these varieties. The apparent increase in the number of pecan varieties susceptible to *F. effusum* is suggestive of the situation for tomato varieties and *C. fulvum*. Whether *F. effusum* has the plasticity of *C. fulvum* in this regard is a matter for additional study. Such work needs to be done with single-spore isolates to substantiate and expand the promising lead on pathogenic adaptation reported by Demaree and Cole (7).

Leaflet disc test.—In the crude *in-vitro* test of host effects on pathogen growth reported here, the great variability inherent in the technique used obscured any individual statistical comparisons that might have been made, forcing comparisons among the groups. The stimulation of germ-tube growth by some leaflet discs and depression by others as compared to water agar controls is not readily explained; however, conclusions from the leaflet disc treatments are drawn by comparing them with each other, not with water agar controls. Differences between "own" and "other" disc treatments averaged 38 μ, and the greatest difference was 101 μ. Greater differences than these would be

Table 5.—Comparisons of germ-tube lengths near susceptible versus resistant leaflet discs.

Conidia from lesions on variety:	Host response				P for comparison
	Germ-tube		Resistant		
	Susceptible				
	Varieties [a]	Germ-tube length (μ)	Varieties [a]	Germ-tube length (μ)	
Squirrel	Sq,B,So	226	W,St	195	$P < 1\%$
Burkett	Sq,B,So	230	W,St	199	$10\% < P < 30\%$
Western	W,So	236	Sq,B,St	215	$1\% < P < 5\%$
Sovereign	So	281	S,B,W,St	212	$1\% < P < 5\%$

[a] See Table 1 for explanation of abbreviations.

Table 6.—Key to 4 races of *Fusicladium effusum* found in Oklahoma.

Source of inoculum	Race no.	Reaction [a] on host variety			
		Squirrel	Burkett	Western	Sovereign
Squirrel	1	+	+	+	+
Western	2	–	–	+	+
Sovereign	3	–	–	–	+
Burkett and Squirrel	4	+	+	–	0

[a] + indicates infection; – indicates lack or only a trace of infection; 0 indicates no data.

desirable. Comparisons, chosen to parallel expected field behavior, were statistically significant near and below the 10% level of probability. If lengths near "own" discs are set as standards and if pathogenicity is assumed when lengths of germ-tubes near "other" discs equal or exceed "own," 16 of the 19 disc-conidia combinations that can be compared with controlled inoculations fit the predicted pattern of pathogenicity from field and greenhouse data.

Leaf extracts and exudates from resistant and susceptible leaves have been found to act differentially on several fungus pathogens. Johnstone (8) found that liquids expressed from apple leaves resistant to *Venturia inaequalis* (Cke.) Wint. were more toxic to *V. inaequalis* conidia than were liquids expressed from susceptible apple leaves. Kirkham (9) extended this work to demonstrate that polyphenolic host metabolites may play a role in the resistance of apple leaves to *V. inaequalis*. Kovács (10) showed that germ-tube lengths of *Cercospora beticola* Sacc. were significantly shorter in water from the leaf surfaces of resistant sugar beets than in water from susceptible leaves.

The pecan leaflet disc test does indicate that diffusible substances that differentially inhibit conidial growth near susceptible as compared with resistant discs are associated with resistance to *F. effusum* in pecan leaflets often enough to suggest more than a chance relationship. Purification of whatever substances may be involved in the differential depression of growth of germ tubes *in vitro* has not been attempted, except to note that they are heat-labile. The data do not indicate whether this differential depression of germ-tube growth is functional in disease resistance.

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INFLUENCE OF ENVIRONMENT ON DISEASES OF TURFGRASSES. I. EFFECT OF NUTRITION, pH, AND SOIL MOISTURE ON RHIZOCTONIA BROWN PATCH

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SUMMARY

A continuous-flow drip-culture system, with silica sand as the support-medium, was used for growing Seaside bentgrass (*Agrostis palustris*) at various levels of nutrition and pH. Postemergence inoculation with *Rhizoctonia solani* was accomplished by adding aqueous mycelial suspensions around the crowns of plants. Disease severity in plants was much greater at high nitrogen levels than at normal or low levels. Low nitrogen gave significantly less disease than a normal level. High or low levels of potassium or phosphorous did not influence disease reaction. Disease severity was greater at a low level of balanced nutrition, however, than at a normal level. At low nitrogen, altering pH did not influence disease reaction; at high nitrogen, disease intensity was significantly greater (1:19) at pH 5.6 and pH 9.0 than at pH 4.0. With normal nutrition, disease was least severe at pH 8.5 and pH 10.0. The influence of 5 soil-moisture levels ranging from field capacity to permanent wilting percentage on disease development was checked by varying the time of irrigation prior to inoculation at each level. Within the soil-moisture ranges studied, no differences in disease development were noted.

In the highly specialized field of turfgrass production, the relations of management practices to disease development is of prime importance. Therefore, observations concerning the correlation between fertility and water management, and disease occurrence are distinctive, both in their numerical magnitude and general dispersal of degrees of positiveness. The present series of investigations aims at more accurate delineation of the role of various environmental factors in the development of diseases of turfgrasses.

Rhizoctonia solani Kühn, the incitant of Rhizoctonia brown patch, parasitizes many of the grass species used as fine turf. The organism is particularly destructive on bentgrass (*Agrostis* spp.). With the turfgrass, *R. solani* is primarily a foliar pathogen; during prolonged hot, humid weather, however, crowns and roots may become severely diseased, with death of the plants resulting.

Several workers have suggested that fertility may influence the development of Rhizoctonia brown patch. It has been observed that turfgrass made succulent by high nitrogen applications may become heavily diseased (6, 7, 10, 11, 12, 15). In addition, Kernkamp et al (8) have reported that predisposition of wheat to infection by *R. solani* was greater in soils deficient in calcium and magnesium and low in pH and in soils completely lacking those elements.

Das and Western (4) reported that the growth of *R. solani* in sterilized soil was increased by adding complete or phosphatic fertilizers or by moderately increasing nitrogen and potassium. In unsterilized soil, the fertilizer amendments most likely to increase disease were the balanced and nitrogen series; potassium and phosphate had no significant effect.

Blair (1) demonstrated that the naturally occurring cellulose-decomposing soil microflora depressed the growth of *R. solani* in soil when excess nitrogen was added.

Hearn (6) showed that *R. solani* occurs in soils with reactions ranging from pH 6.0 to 8.2, with pH 7.0 being optimum for growth. Blair (1) observed that the fungus grew within the range of pH 5.8 to 8.1, and that a neutral soil reaction appeared optimal. Montieth and Dahl (10) found that the fungus grew between pH 3.6 and 8.2, with optimum growth between pH 5.8 and 7.0. Kernkamp et al (8) reported little effect from soil pH except when soil reaction is at such a level that it interferes with the availability of certain nutrients. Sprague (13) showed that pH may effect the growth of bentgrass by altering the ratio of top-growth to roots. Nitrate additions to a solution of pH 4.5 greatly stimulated top growth without markedly changing root development, but nitrate added to a solution at pH 6.5 reduced root growth by 35%.

Blair (1) has reported that within a soil moisture range of 30–80% saturation capacity, *R. solani* grows best at the lowest moisture level. Similar results have been reported by Das and Western (4), who found that Rhizoctonia root rot of lettuce was greatest on plants grown at 40% saturation capacity as compared with those produced at 100%.

Materials and methods.—Seaside bentgrass (*Agrostis palustris* Huds.) was grown on quartz sand in 1-gal. glazed crocks fitted with an automatic siphon to allow drainage when the nutrient solution was ½ in. from the surface. A plastic window screen of the inside dimensions of the crock, placed on the sand before the final ½ in. of sand was added, allowed easy removal of the plants at completion of the experiment. Seed to produce about 200 plants was sown in each crock, and distilled water was applied periodically until the plants were established. Greenhouse temperature during the course of the experiments was maintained at 70°F. Modified Hoagland's solutions were then added to the crocks by means of the drip-culture method as described by Gallegly and Walker (5). The formulae of the various modifications of Hoagland's solutions used are given in table 1 of Gallegly and Walker (5). The concentration of the major salts in the basal solution (1.0 H) was modified to 0.1 (0.1 H) and to 3 times (3.0 H). The basal solution was also modified to contain 0.1 (Low) and 3 times (High) the concentration of nitrogen, phosphorus, and potassium ions, in the basal solution. The concentration of

minor elements was the same in all solutions.

The effect of pH was checked with the basal, high-nitrogen, and low-nitrogen solutions. The pH was altered by adding HCl or NaOH to the solutions. The basal series was conducted at pH 4.0, 5.6, 7.0, 8.5, and 10.0. The nitrogen series contained solutions at pH 4.0, 5.6, 7.0, 8.0, and 9.0.

In each experiment, an individual treatment consisted of 20 crocks (4 replicates of 5 crocks each).

Rhizoctonia solani, isolated from Colonial bengrass (*Agrostis tenuis* Sibth.), was grown on potato-dextrose–yeast agar in Petri plates until the agar surface was completely covered. The contents of 250 Petri plates were then chopped in a Waring blender and brought to 10 liters with distilled water. After 100 ml of the mycelial suspension was poured over the plants, each crock was covered with a plastic bag. The bags remained in place until the fungus was established. About 3 weeks after inoculation, the edge of the plastic screen was lifted, the roots below the screen were cut with a knife, and the individual plants were counted. Disease incidence was recorded as per cent dead plants per treatment. All data were subjected to analysis of variance.

The influence on disease development of soil moisture stress in the readily available range, field capacity (FC) to permanent wilting percentage (PWP), was studied by varying frequency of irrigation (3, 14).

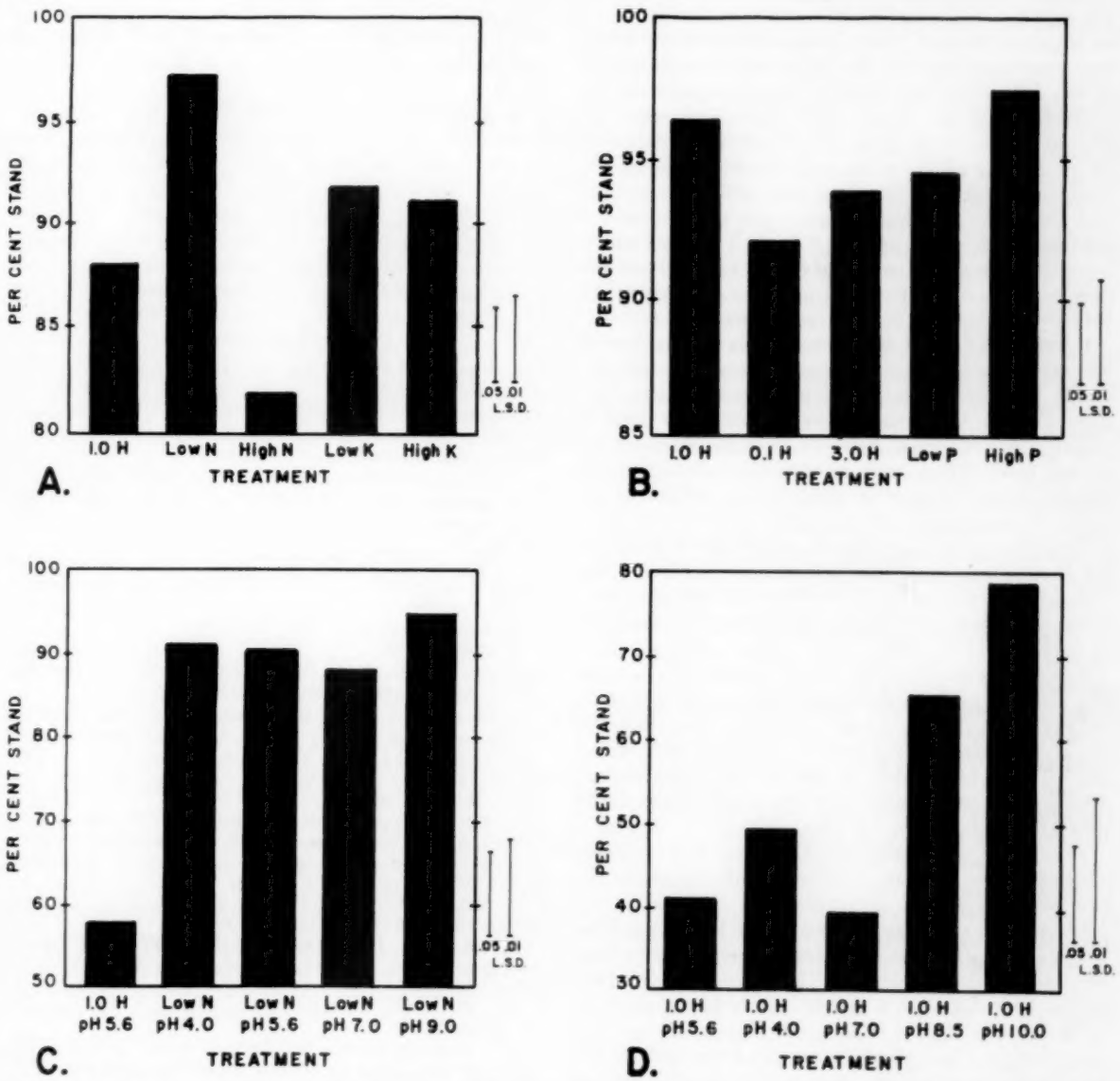

Fig. 1.—Influence of nutrition and pH on the development of Rhizoctonia brown patch of Seaside bentgrass. **A)** Effect of nitrogen and potassium. **B)** Effect of unbalanced solutions and phosphorous. **C)** Interaction of pH and low nitrogen. **D)** Interaction of pH and 1.0 H solution.

Hagerstown Silt Loam, with a moisture equivalent (2) of 24.5%, and a PWP, as determined by the use of dwarf sunflowers (9), of 10.1%, was used throughout this phase of the investigation.

One hundred circular tarred non-porous containers measuring 11×7.5 cm, were filled with a standard weight of soil of known initial moisture content and seeded to Seaside bentgrass. Soil in all containers was maintained near FC, by weight, until root permeation had occurred. Then 5 groups of 20 containers each were selected to use for the following moisture-stress studies: FC, ¾ FC, ½ FC, ¼ FC, PWP.

For the next 2 weeks the plants of each group were allowed to extract moisture to the designated soil moisture stress as determined by weight of the system (14), and were then irrigated to the predetermined FC weight. At the end of this period, when entries of each group reached their designated moisture stress, they were inoculated in the following manner: A core of soil 4 cm deep was removed from the center of the container with a 15-mm cork borer. A standard amount of inoculum, prepared by growing *R. solani* on an autoclaved rye-grain medium and grinding the fungus-permeated grains in a food mill, was tamped into the hole, and the container was covered with a pre-moistened plastic bag for 4 days. Two weeks from inoculation, readings were taken on the basis of per cent dead plants in each soil moisture treatment. All data were subjected to analysis of variance.

Results.—*Effect of nutrition.*—Disease development was more severe on plants grown at high nitrogen nutrition than on plants grown at normal nitrogen fertility (Fig. 1-A). Disease was significantly less on plants at low nitrogen, however, than on plants at the normal level. There were no significant differences in development of disease on plants grown at high or low levels of potassium or phosphorous. Plants were more susceptible to infection by *R. solani* when grown at 0.1 H nutrition than when grown at the 1.0 H level. Plants subjected to 3.0 H fertility did not show any increase in disease proneness over those grown at 1.0 H nutrition (Fig. 1-B).

Effect of pH.—The pH levels tested at low-nitrogen fertility gave no significant differences in disease ratings (Fig. 1-C). Under high nitrogen, however, disease was significantly greater (1:19) at pH 5.6 and pH 9.0 than at pH 4.0. In the 1.0 H series, disease development was significantly less at pH 8.5 and 10.0 than at pH 4.0, 5.6, or 7.0 (Fig. 1-D).

Effect of soil moisture.—The five soil moisture stresses tested gave no significant differences in disease severity.

Discussion.—The incidence of large brown patch on Seaside bentgrass can be altered sharply by varying pH and nutritional levels. The alteration, however, cannot be reduced to a simple, single factor reaction, as has been attempted in the past. The results indicate instead that plant vigor is not as important as the nutritional combination that produced the rate of growth in question. For example, vigor reduced by low nitrogen nutrition, with normal phosphorus and potassium levels, reduced disease severity, but vigor reduced by low nitrogen with low phosphorus and potassium (0.1 H) accentuated disease proneness.

High nitrogen levels, with normal phosphorus and potassium nutrition, increased vigor and increased disease development, but the heightened disease proneness was offset if phosphorus and potassium were increased concurrently (3.0 H).

The general complexity of the over-all problem of nutrition, plant vigor, and proneness to large brown patch is further illustrated by results in the series with varied pH. Under low nitrogen nutrition, pH had no apparent influence on disease development. With normal, balanced nutrition, disease development was greater in the acid range. At high nitrogen levels, however, disease was more severe in the basic range. This interaction of factors provides a possible explanation of general lack of agreement in the literature on soil fertility and the development of Rhizoctonia brown patch.

In interpreting the results of the soil moisture series, it should be remembered that the stresses studied were in the readily available range. The negative results obtained in this investigation, then, do not preclude the possibility that stresses approaching saturation capacity might influence the development of Rhizoctonia brown patch.—Department of Botany and Plant Pathology, The Pennsylvania State University, University Park.

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INTRA- AND INTER-REGIONAL MOVEMENT OF UREDOSPORES OF BLACK STEM RUST IN THE UPPER MISSISSIPPI RIVER VALLEY

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SUMMARY

Two recently developed devices, the rotobar sampler and the airplane rod sampler, were employed to study the extent and concentration of stem rust uredospores in air. The rotobar sampler, relatively simple, inexpensive, sensitive, and highly efficient in sampling airborne stem rust uredospores, measures airborne spore concentrations at a particular location. The airplane rod sampler measures the uredospore content of the air between various locations at various altitudes. It was used over the northern Mississippi and Red River Valleys during June and early July, 1957. In artificially inoculated wheat plots the production of stem rust spores was greatest when the wheat was in the soft-dough stage of growth, considerably before the rust reached maximum severity. Greatest production coincided with the peak in the number of new pustules. The degree and extent of local downwind spore travel depended on wind velocity, time of day, number of spores produced by the infested plot, and location of the pustules on the plant. Uredospores were not found in rain samples collected between June 10 and July 6, 1957, at Crookston, Minnesota, although a natural spore shower fell on July 1 and 2.

In the study of any parasitic plant disease the nature and speed of dissemination of the causal organism are considerations of prime importance. Interregional spread of wheat stem rust (caused by *Puccinia graminis* Pers. *tritici* Erikss. & E. Henn.) is through rapid and extensive dissemination of uredospores, as well established by Stakman (5) and others. Thus, the northward movement of uredospores from the hard red winter wheat region of Kansas and Oklahoma has been observed under some conditions to cause severe stem rust epidemics in the spring wheat region of the Dakotas, Minnesota, and Canada.

In contrast, local spread or movement of stem rust in epidemic proportions from one part of a field to another, to adjacent fields, or to other fields in the same region has been found to be very limited. Lambert's field observations (4) indicate that a considerable quantity of inoculum must accumulate before stem rust migrates in perceptible amounts from one field to another.

The present investigations were designed to determine the factors affecting local and interregional dissemination of wheat stem rust uredospores. Daily air samplings made possible the detection of natural spore showers and their spore concentrations before the liberation of uredospores in fields that had been artificially inoculated.

Gregory (2) found that sampling efficiency of gelatine-coated metal rods is, within limits, directly proportional to wind velocity and inversely proportional to the diameter of the rod. Following Gregory's discoveries the rotobar and airplane rod samplers were developed. The rotobar sampler rapidly rotates a bar through air, picking up spores much as does a stationary coated rod in a wind of moderately high velocity. The coated rod of the airplane rod sampler likewise moves rapidly through the air, though it is stationary with respect to the aircraft. Both samplers are further described in this paper. (The rotobar sampler was originally developed and used in Fort Detrick, U. S. Army Chemical Corps, by Messrs. R. M. Acker and R. W. Hartmeyer, now of the Chemical Corps. They are presently preparing a paper with a more detailed description of the sampler. It was tested and improved by Dr. W. A. Perkins, Aerosol Laboratory, Stanford University. Mr. Hartmeyer suggested the use of the rod in aerial spore sampling, and Mr. N. F. Wells and the writer developed the airplane rod sampler.)

Materials and methods.—The rotobar sampler was used in these studies to measure the concentration of stem rust uredospores at plant height in a particular location for a given period. The airplane rod sampler, in contrast, was used to measure the concentration of spores between two locations for a particular time-period and altitude.

In both samplers, brass rods, 1.59×1.59 mm in cross section and coated with a sticky glycerine jelly, are used to trap the spores from the sampled air. (Rods with non-corrodible surfaces would be preferable since brass rods were quickly corroded by substances in the glycerine jelly, even when coated with lacquer.) The volume of air sampled is a function of the area of the leading face of the adhesive-covered rod, the relative velocity at which the rod is moved through the air, and time.

The rod coating is essentially the same as that employed by Gregory (2). It consists of: 6 g gelatine, 21 g glycerine, 18 cc distilled water, and 0.45 g phenol.

A rotobar unit consists essentially of a rotobar and

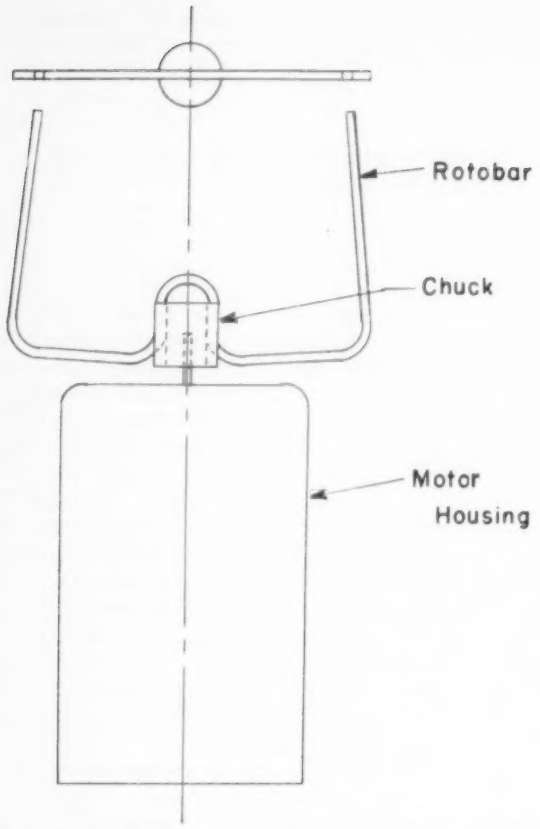

Fig. 1.—Diagram of an improved model of a rotobar assembly.

a small electric motor. The motor rotates the rotobar (brass rod) at about 2500 rpm when connected to a battery circuit (Fig. 1). Each of the upright arms of the rotobar is coated by dipping it in melted glycerine jelly.

The rotobar sampler, in addition to its simplicity, is highly efficient (over 90%, according to a complete description of the rotobar sampler by Dr. W. A. Perkins, Aerosol Laboratory (Stanford) in: The Roto-rod Sampler; Second Semiannual Report, March 1957–August 1957, Chemical Corps Research and Development Program) for collection of particles in the 20-μ range, sensitive to low concentrations of particles (sampling rate, 120 L of air per minute). Only a low-power source (12-volt dry-cell battery) is required. Cost of the unit was less than $10.00.

Fig. 2.—Sequential sampler mounted in the center of a wheat plot.

A sequential rotobar sampler, designed for continuous sampling, is made up of 24 rotobar units, each being run for one hour. The 24 units are mounted on a steel ring (Fig. 2), each connected and controlled by a clock-and-battery mechanism.

The airplane rod sampler consists essentially of a glycerine-jelly-coated rod exposed from a small single-engine aircraft at various locations and altitudes to detect spores and measure their concentration.

To allow for simple exchange of sampling rods and at the same time ensure that they are exposed outside propellor wash and any complicating air turbulence, the rods are fastened in a square dowel and pushed from inside the plane through an 8-foot-long square aluminum tubing to the air outside. The dowel serves to hold the rod firmly in position, preventing it from touching the walls of the tube through which it is pushed, and serves as a stop when the end of the tube is reached. Finally, it keeps the rod from twisting, ensuring that one face constantly faces the wind. The square tubing protrudes through the plywood cabin door and is fastened near the end to the wing strut (Fig. 3).

To facilitate the handling of the coated rods in the plane before and after the sampling runs, one end is

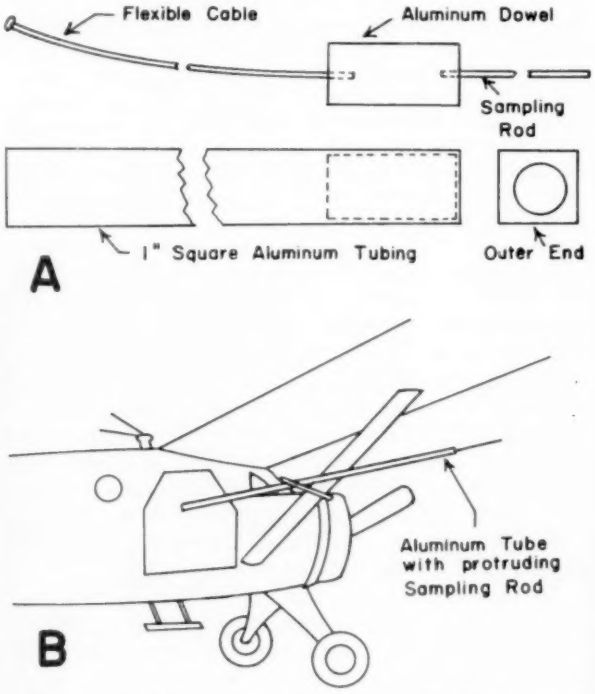

Fig. 3.—Aerial spore sampling device. **A)** Airplane rod sampler. **B)** Sketch of the sampler mounted on an L-20 Army aircraft.

inserted in a cork, and, in turn, the cork and rod are inserted in a test tube.

The airplane rod sampler is simple to construct, install, and operate. It has a high sampling rate (440 L per minute with a 10-cm-long rod at an air speed of 100 mph). Cost of the unit plus installation was less than $20.00.

Counts of the stem rust uredospores trapped by impaction on the rod are made by direct microscopic examination under vertical illumination. It is advisable to delay the counting at least an hour to allow the shrunken uredospores to swell up to full size on the glycerine jelly.

Aerial sampling in the Red River and Northern Mississippi Valleys.—One of the earliest attempts to sample spores in the upper air was the work of Stakman et al (6), who exposed hand-held vaseline-coated microscope slides while seated in the cockpit of a plane and slides held in a boxlike mechanical spore trap mounted on the wing. The hand-held slides proved more efficient than those exposed in the mechanical spore trap, which is reasonable in light of Gregory's recent findings (2, 3) that, within certain limits, rods with small diameters were more efficient than rods with large diameters. Stakman's mechanical spore trap, with its considerable bulk, would thus be a less efficient spore trap than a single hand-held slide.

An isokinetic, pump-operated sampler using millipore filters proved unsatisfactory. The equipment is bulky, relatively complicated, and subject to numerous breakdowns. Furthermore, assessing stem rust uredospores on millipore filters is a tedious task since the spores are not deposited uniformly.

The airplane rod sampler, because of its extreme simplicity in its design and operation and high air-sampling rate, appears to be an excellent spore trap for aerial sampling. Its efficiency should not differ markedly from that of the rotobar sampler, which operates on essentially the same principle.

Results.—A number of aerial sampling runs were made from June 21 to July 2, 1957, over the Red River and northern Mississippi valleys to determine the nature and extent of interregional spore showers during that period. The runs were made with the airplane rod sampler mounted on a L-20 Army aircraft. The results are in Table 1. The flight on June 21, made at

Table 1.—Spore concentrations measured at various altitudes and locations measured by airplane rod sampler, June–July, 1957.

Date	Rod no.	Location	Altitude	Time	Spores Per m[a]
Jun 21[a]	1	Crookston, Minn. to Fargo, N. D.	1000	1325–1355	0
	2	Fargo to Sisseton, S. D.	3500	1440–1530	0
	3	Sisseton to Brookings, S. D.[b]	3600	1530–1630	
	4	Brookings to Sioux Falls, S. D.[b]	3400	1630–1700	
	5	Sioux Falls to Hawarden, Iowa[b]	2900	1700–1730	
	6	Hawarden to Sioux City, Iowa[b]	2500	1730–1745	
Jun 22	7	Sioux City to Lincoln, Nebr.[c]	4000	0830–0930	0
	8	Lincoln to Ft. Riley, Kansas[b]	4000	0930–1030	
Jun 24[d]	9	Ft. Riley to Kansas City, Kansas	6000	1140–1240	17
	10	Kansas City to Sidney, Iowa	6000	1240–1340	13
	11	Sidney to Omaha, Nebr.	6000	1340–1355	6
	12	Omaha to Sioux City, Iowa	2500	1355–1440	5
	13	Sioux City to Brookings, S. D.	2500	1540–1640	0.1
	14	Brookings to Fairmont, N. D.	2500	1640–1740	0
	15	Fairmont to Fargo, N. D.	2500	1740–1815	0
Jun 29[e]	16	Crookston, Minn to Fargo, N. D.	5500	0800–0830	0
	17	Fargo to Minneapolis, Minn.	5500	1045–1215	0
	18	Minneapolis to Pierre, S. D.	5500	1230–1300	0
	19	Minneapolis to Pierre, S. D.	5500	1300–1340	0.2
	20	Minneapolis to Pierre, S. D.	5500	1340–1400	0.6
	21–24	Minneapolis to Pierre, S. D.	5500	1400–1545	0
	25	Pierre to Aberdeen, S. D.	6000	1650–1800	0
	26	Aberdeen to Fargo, N. D.	6000	1830–1900	0
	27	Fargo to Crookston, Minn.	5000	1900–1930	0
Jul 1[f]	28	Crookston to Fargo, N. D.	2500	1035–1130	25
	29	Fargo to Valley City, N. D.	2000	1230–1300	33
	30	Valley City to Fargo	2000	1300–1320	17
	31	Fargo to Valley City	2000	1320–1350	29
	32	Valley City to Jamestown, N. D.	5000	1350–1420	7
	33	Jamestown to Magnolia, N. D.	5000	1420–1450	6
	34	Magnolia to Fargo to Crookston	5000	1450–1540	7
Jul 2[g]	35	Crookston, Minn.	5000	1420–1426	0
	36	Crookston, Minn.	3000	1435–1445	0

[a] 20–50 mph south winds.
[b] Spores present but rods dirty.
[c] Just after a rainstorm.
[d] 15–30 mph south winds.
[e] Winds in the west and northwest.
[f] Strong south wind.
[g] Wind northwest.

an altitude of 3500 feet, indicated that the northernmost limit of stem rust spore travel at that time was somewhere south of Sisseton, South Dakota. The June 24 flight indicated that the northernmost limit was south of Brookings, South Dakota.

The flight altitudes were selected by the pilot on the basis of local regulations, weather conditions, and convenience. Samplings at these altitudes are assumed to give a representative picture of stem rust spores in the atmosphere since Stakman et al (6) found that stem rust spores were trapped at all altitudes up to 11,000 feet, above which their numbers dropped off very rapidly.

On June 29 a triangular course was flown between Crookston, Minnesota; Minneapolis, Minnesota; and Pierre, South Dakota. West and northwest winds prevailed during the flight. Low concentrations of spores, indicating the northernmost limit of uredospore movement, were picked up at or near central South Dakota and Southern Minnesota. Thus, we can be reasonably certain that the northernmost limit of stem rust spread passed through central South Dakota between 21 and 29 June.

The limit of spore travel was much further north on July 1. Aerial sampling on that date shows that the limit had reached and probably passed to the north of Fargo, North Dakota. This was confirmed by sampling devices on the ground at Crookston, Minnesota, as discussed later herein.

Stem rust spores in rain samples.—To determine whether rains were instrumental in carrying down stem rust spores carried northward by the winds during this season, rain samples were collected and assayed for spore content.

The rain was collected in "Tru-Check" rain gauges, which are wedge-shaped, with a large opening at the top, and constructed of Plexiglass with a graduated scale to allow accurate measurement of small quantities of rain.

The gauges were mounted on a bracket one meter above the ground. Six were placed on the lawn of Hill Building, Northwest School of Agriculture, University of Minnesota, and six at T Plot (described in the next section). Both locations are in the vicinity of Crookston, Minnesota. The gauges were washed daily to minimize the amount of dirt and other materials that might interfere with spore counting.

To determine the number of spores present, the rain collected in the gauges was passed through millipore filters under vacuum. The filters were examined to determine whether spores were present.

Results.—At no time between June 10 and July 6 were stem rust spores detected in the rain samples, although rains fell on June 13, 14, 15, 17, 22, 23, 25, 26, and 27, and a natural spore shower fell on July 1 and 2.

Detection of natural spore showers and measurement of spore concentrations.—A sequential rotobar sampler was mounted at one-meter height in the center of Plot T and operated continuously from June 10 to July 10, 1957, to determine the time of arrival of a natural spore shower or of spores blown in from artificially inoculated plots at the Northwest School of Agriculture.

Plot T (on the farm of Mr. D. Lien, and set up through the cooperation of Mr. D. G. Fletcher, Rust Prevention Association) was about 6 miles west-southwest of the Northwest School of Agriculture and adjacent to Red Lake River between Crookston, Minnesota, and Grand Forks, North Dakota. It was of about 3 acres and planted to Baart wheat, a variety susceptible to most races of wheat stem rust.

Results.—From June 10 through 30 no stem rust spores were collected by the rotobars and the wheat remained free of stem rust. On July 1 and 2 a strong, steady south wind brought an abundance of stem rust spores. Following weather was favorable for spore germination.

On July 9 stem rust pustules were noted for the first time in Plot T. The pustules occurred uniformly throughout the entire field, mostly on the sheath and blade of the top leaf. Prevalence was 20%. There seems to be no question that the infection resulted from inoculum arriving with the preceding spore shower.

Since there was no longer any way to differentiate between spores from other areas and spores from the infected plot, sampling was discontinued.

Seven collections of rust samples were taken at Plot T for race identifications. The only sample identified successfully was of Race 56. It is unlikely that Race

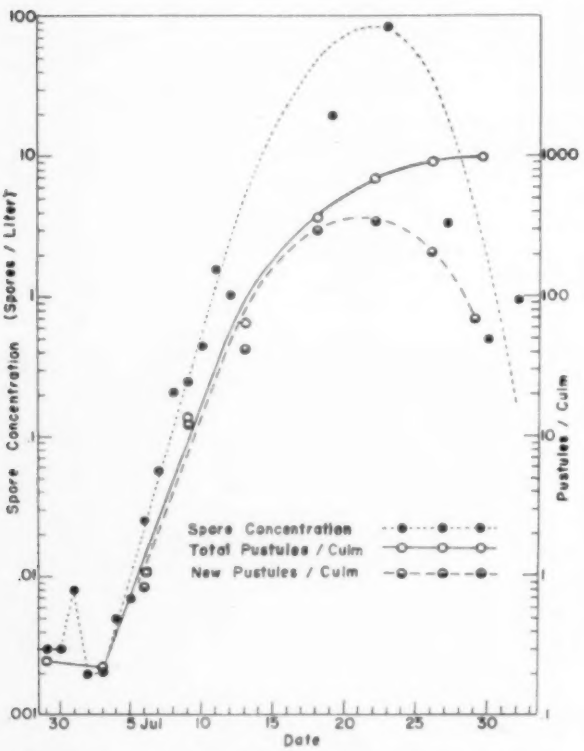

Fig. 4.—Graph showing relationship between spore concentration over Plot O, number of pustules per culm, and number of new pustules per culm.

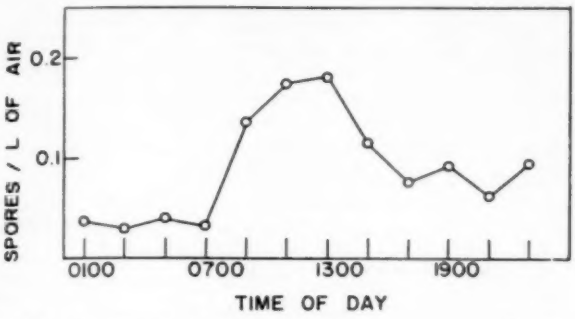

Fig. 5.—Graph showing average spore concentration for 1-hour periods over Plot O from July 4 through 9, 1957.

15B came in at this time, since the susceptible variety, Lee, planted at the Northwest School of Agriculture, showed no rust on July 9.

Production of stem rust spores in artificially inoculated wheat plots.—A sequential rotobar sampler was mounted at the level of the tops of the plants in the middle of Plot O from June 29 through August 1 to determine the concentration of uredospores over the artificially inoculated plot. Plot O, of one acre, was on the farm of the University of Minnesota's Northwest School of Agriculture, Crookston, Minnesota. It was planted to Baart wheat on May 3, 1957, and on June 15 was inoculated by an aerial dissemination system with Race 56 of wheat stem rust.

Fewer personnel and a high concentration of spores after July 12 limited operation of the spore samplers to 10-minute and 1-minute periods between 9:00 A.M. and 4:00P.M. A 4-foot-wide pathway and a circular area 8 ft in diameter in the center of the plot were cut out to mount the sampler and provide free access through the plot to the sampler.

The plot was divided into 9 sub-plots, in each of which independent appraisals of rust intensity or severity were made by 4 men. The results were averaged to give a picture of rust development throughout the season.

Results.—The average daily concentration of stem rust spores recorded over Plot O is plotted in Fig. 4, together with rust development data and the number of new pustules being formed.

Plotting spore concentration against time gives a modified sigmoid growth curve. Peak spore production occurred on or about July 23, when the wheat was in the soft-dough stage. The curve for the number of pustules per culm follows that of spore concentration rather closely. Spore concentration fell off sharply after July 23, however, while pustules per culm continued to rise. The reason is evident when that curve for the number of new pustules is plotted against time. In all probability, most of the spores were produced by the new pustules and new areas of pustule enlargement. Thus, the curve for spore concentration would logically follow closely the curve for the number of new pustules rather than the curve for the total pustules. New growth on enlarging pustules would be equivalent to new pustules as far as spore production is concerned, and would be accounted for in the rust severity estimate.

The hump in the spore concentration curve for Plot O on July 1 was doubtless due to the natural spore shower that blanketed that area on that date. The plateau or negative slopes to the spore and pustule concentration curves early in the season is due to occurrence of original inoculation and infection within a period of a day or two and no further inoculations or infections until at least 10 days later, when pustules from the original infection began spore production. Thus, the number of pustules and spore production remained stable for at least 10 days. The apparent decrease in number of pustules during this period may reflect a loss of some of the oldest infected leaves or a coalescence of some of the pustules.

Although spores are present over infested plots throughout the entire day, the largest numbers are trapped about mid-day (Fig. 5, 6). The hourly spore concentrations are extremely variable and appear subject to rapid change. Fig. 6, a graph of spore concentrations during a single day (July 9), illustrates these points.

Humidity and wind velocity seem to be important factors affecting spore dissemination. Spores gain or lose moisture rapidly, and, under conditions of high humidity, are heavier and perhaps stickier during the night than during the day, thus requiring air currents of higher velocity to make them airborne. The heavier spore would also tend to settle out of the air more

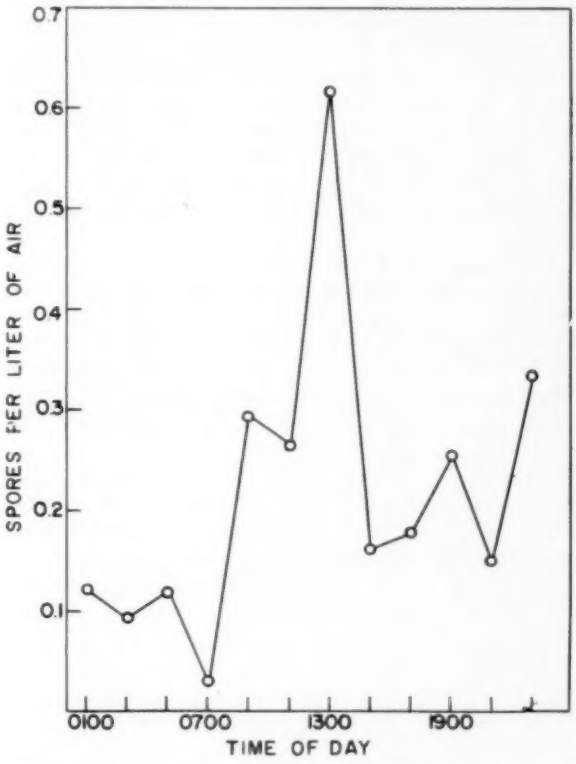

Fig. 6.—Spore concentrations over Plot O on July 9, 1957.

Table 2.—Concentration of stem rust spores in the air downwind from Plot O, Crookston, Minnesota, 1957.

Sampler height	Distance downward	Concentration (spores per m³ of air)					
		July 2 2–3 p.m. wind NW 6–8 mph, clear, 82°F	July 5 3–4 p.m. wind W 9–12 mph, partly cloudy, 79°F	July 5 9–10 p.m. wind W 2–4 mph, clear, 64°F	July 6 10–11 a.m. wind S 5–7 mph, clear, 73°F	July 8 2:45–3:15 p.m. wind NW 12–16 mph, clear, 76°F	July 10 3:20–4:20 p.m. wind SW 4–6 mph, clear, 87°F
26 in.	Over plot	10					
10 in.	Adjacent	8					
1 m	Adjacent	3	7	0.3	2.5		
4 m	Adjacent	0	1	0	0.5	131	17
1 m	5 m		4	0	1.2		
1 m	10 m		6	0	1.5	413	
1 m	20 m		2		0.3	199	19
1 m	40 m	0	1.5		0.8	106	
4 m	40 m	0	0.2		0	156	10
1 m	80 m					65	
4 m	80 m					51	
1 m	Upwind check				0	0	1.2

rapidly. Higher or more turbulent surface winds during the day may also account for higher spore concentrations. Winds of variable velocity, night or day, would cause variable spore concentrations.

Local spore dissemination.—Rotobar samplers were mounted on poles of various heights and placed at various distances from the downwind edge of Plot O at various times between July 2 and 10 to determine the degree of spore travel from the point of origin under varying meteorological conditions.

Results.—The height that the spores rose above the plot and the distance traveled were governed by wind velocity. The data in Table 2 clearly show the effect of wind velocity on local spore travel.

On July 2, only first-generation pustules were present on the plants. The highest pustules on the plant were on the second and third leaves. Average elevation of a large number of the highest pustules on the infected culms was 13 in. when plant height was 26 in. Thus, the pustules were well below the tops of the plants.

The foliage was dark green at that time, and the sun was bright and hot though there was no visible evidence of any appreciate thermal air currents. Very little upward or downwind travel of spores was noted.

On July 6 and 10, when the pustules were much more numerous and located throughout the plant cover, spores were detected much further downwind even with low wind velocities.

On July 8, when the wind was 12–16 mph, spore count downwind was about 10 times that on July 10, when wind velocity was 4–6 mph. The temperature was 11°F higher on July 10 than July 8, and might be expected to result in higher thermal air currents. Furthermore, spore production for the plot was higher on the later date. Greater downwind travel on July 8 is thus clearly due to greater wind velocity.

It is evident that higher-velocity winds carry the spores not only further but higher. That spores are carried higher into the air in winds of higher velocity is in agreement with the findings of Best, as summarized by Sutton, p. 253, on the values for vertical gustiness as affected by wind velocity (6). At a fixed height of 2 meters above grassland, he found that the mean value for vertical gustiness increases with mean wind velocity during lapses and inversions.

Discussion and conclusions.—It has been pointed out that spore production in Plot O was maximum when the wheat was in the soft-dough stage. The hardening and drying of the tissues of the leaves and stems that follow this stage would result in fewer new infections and consequently fewer new pustules. Peak spore production might occur before the soft dough stage if initial infection occurred earlier or if conditions were more favorable for rust development than in these tests. If the wheat plants were severely stunted by rust in the early stages of growth, spore production might not be as high as was found in these studies, because plant surface area available for rust development would be smaller.

One might ordinarily think that spore production is maximum when rust severity is maximum. Fletcher et al (1) believe that spores that fell on North Dakota, northwestern Iowa, western Iowa, and western Minnesota between June 24 and 28, 1952, came from central Oklahoma and south-central Kansas, where combine operations were believed to have dispersed spores into the air. This may have been so, and, if so, most of the spores must have been produced at least 2–3 weeks earlier, before the wheat tissues had hardened and dried. It would be doubtful if such spores retained much of their viability by the time they were airborne, since work by Kingsolver et al at this laboratory demonstrated that stem rust uredospores, exposed to only 45 minutes of dew followed by a dry interval of one hour, were drastically reduced in viability. It appears more likely that the source of spores was much closer, probably central Nebraska, where wheat was probably in the soft-dough stage and rust spore production near its maximum.

The data presented and discussed herein provide an explanation for the apparent anomaly between the rapid and extensive interregional spread of wheat stem rust and the slow and limited local spread of the same

disease.

Uredospore production was about 40,000 times as great on July 23 as on July 3, when production was minimum (Fig. 4). Furthermore, on July 3 the highest pustules were midway between the ground and the top of the plants. At the later date there were many pustules on all aboveground parts of the plant, including the head. Pustules formed on the earlier date, being so deep within the plant cover, were much less apt to be brushed by winds lifting and carrying away uredospores than were later pustules at or near the plant tops. The ratio of number of pustules on the lower half to the number on the upper half decreased as the plants approached maturity.

Actual determinations of uredospore concentrations downwind from an infested plot show practically no downwind travel of spores in winds of 6–8 mph on July 2, whereas measurable concentrations were noted 40 meters downwind from the plot on July 6 in winds of 5–7 mph. The reason is undoubtedly the greater spore production and higher location of pustules on the plant on the later date.

Thus, conditions are generally not favorable for rapid and extensive spread just after the rust is first established. Later, after spore concentrations are higher and a greater proportion of the pustules are located at or near the top of the plant cover, conditions are much more favorable. With each passing week, however, the period available for rust to increase to damaging proportions from a new infection in local wheat fields becomes shorter as the wheat approaches maturity date. In the distant, more northerly fields, the rust has more time to increase since the wheat there is at an earlier stage of growth than the wheat in the local fields.

A high-velocity southerly wind passing over a highly infested source field at the early-dough stage of growth could pick up great quantities of uredospores and carry them great distances to northern wheat fields, where the wheat is still in the tillering or early-joint stage. Thus, conditions would favor an epidemic.—U. S. Army Chemical Corps, Fort Detrick, Frederick, Maryland.

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STUDIES ON ROOT ROTS OF RED CLOVER IN WISCONSIN

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SUMMARY

The pathogenicity of 49 fungus isolates from diseased red clover roots in Wisconsin was studied in the greenhouse. Pure cultures were tested in white silica sand, steamed soil, fumigated soil, and 5 naturally infested field soils. The isolates were generally more pathogenic on seedlings than on older plants. A wide range in pathogenicity among isolates of some species was demonstrated. Unclipped plants escaping seedling infection showed little more than a trace of root-rotting after 5 months' growth. Some stunting occurred, however, as indicated by dry-weight determinations. After preliminary tests the number of isolates was reduced to 10:3 of *Fusarium oxysporum*, 3 of *F. solani*, and 1 each of *F. roseum*, *F. moniliforme*, *Gliocladium roseum*, and *Rhizoctonia* sp. Clipping foliage at frequent intervals weakened plants and increased susceptibility to root rots. The amount of clipping that plants could tolerate was greatly influenced by their rate of growth, which was influenced by temperature, light, and other environmental conditions. Twelve-week-old plants recovered less rapidly and developed more disease than 8-week-old plants when given similar clipping treatments. Clipping was found to be a useful technique for predisposing red clover to attack by weak pathogens. More disease developed at high than at low temperatures, and more in some soils than in others. Adding pure cultures of 10 isolates to naturally infested field soils from 5 locations had little effect on disease severity of unclipped plants. When plants were weakened by clipping, some isolates reduced disease severity in certain soils, some increased severity, and some had no apparent effect.

Red clover (*Trifolium pratense* L.) is a perennial, though in humid areas of the United States it rarely survives more than 2 years. Stand deterioration of red clover is an old problem (5). It was reported in Europe at least as early as 1669 (5) and in the United States by 1747 (5). In England it was serious enough that workers at the Rothamsted Experiment Station began to study the problem in 1849. Pieters (15, 16), Fergus (4, 5), Valleau (5), and Selby (17) were

among the early workers in the United States who emphasized its importance and suggested causes and means of control.

Many early stand failures were undoubtedly the result of attempts to grow red clover in unsuitable soils and the use of unadapted varieties, but even today there is a continuous decline in plant numbers in virtually all fields. Kilpatrick and Hanson (10) estimated losses of 39–52% in first-year stands in Wisconsin. Smith (20), also working in Wisconsin, found that 60% of the plants that survived their first summer failed to survive their first winter, and that a large proportion of those remaining died during the following summer. In Ohio, stand losses of 95% in a single growing season have been reported in individual fields (19). Several factors contribute to stand deterioration, but disease has been a major factor, particularly root and crown diseases (3, 5, 8, 13). Kilpatrick et al (11) have discussed most of the fungi reported as incitants.

Fusarium spp. have probably been isolated from diseased red clover roots more frequently than any other fungi. In 1909 Selby and Manns (18) isolated *Fusarium roseum* Lk. from clover roots in Ohio. In 1921 Clayton (2) found *Sclerotinia trifoliorum* Eriks. and *Fusarium* spp. associated with stem and root rots of clover in western and southwestern Ohio. About the same time and also in Ohio, Selby and Thomas (19) reported isolating *Fusarium* sp. consistently from rotting clover roots. Young (22), in 1923, reported that *Fusarium* spp. were by far the most common and widespread causes of root rots of clover in Ohio. Young found *Gibberella zeae* (Mont.) Petch (*Fusarium roseum*) the most common species. In 1924 (23) he isolated numerous *Fusarium* spp. from clover roots but did not name the species. Fergus and Valleau (5) found *Fusarium moniliforme* Sheldon, *F. solani* (Mart.) App. & Wr., and an isolate resembling *F. oxysporum* Schlect. consistently associated with clover root rots in Kentucky. Horsfall (9) isolated several *Fusarium* spp. from rotted crowns and roots of red clover in New York. Allison (1) isolated several species of *Fusarium* from red clover roots in Wisconsin but did not identify them. Kilpatrick and Hanson (10) reported *F. oxysporum* Schlect. emend Snyd. & Hans., *F. solani* (Mart.) App. & Wr. emend. Snyd. & Hans., *F. roseum* (Lk.) emend. Snyd. & Hans., and *F. moniliforme* (Sheld.) emend. Snyd. & Hans. as being commonly associated with the diseased roots of this crop. The same 4 species were reported by Crall (3) from rotted clover roots in Iowa. Kreitlow and Hanson (13) consistently isolated *F. oxysporum* and *F. solani* from red clover roots in Pennsylvania. Neuweiler (14) reported *F. trifolii* Jacz. as pathogenic on red clover roots in Switzerland and Russia. It is evident that *Fusarium* spp. occur in close association with rotted red clover roots wherever the crop is grown.

The objectives of the present investigation were 1) to obtain further information on the pathogenicity of some of the fungi commonly associated with root rots of red clover in Wisconsin, 2) to determine the effects of clipping foliage on susceptibility to these diseases, and 3) to develop better methods of testing pathogenicity of root pathogens. Since it has been demonstrated that many of these fungi can attack clover seedlings (7, 12), emphasis was directed toward determining the effect of the fungi on post-seedling plants. The results were abstracted in 1954 (6).

Materials and methods.—All plants originated from a seed lot of Wisconsin Mildew Resistant red clover produced in Prosser, Washington. This seed lot was remarkably free of injury and fungus infection. All seed was surface-sterilized for 5 minutes in a 0.5% sodium hypochlorite solution. In experiments not involving transplanting, the seed was sown directly into the artificially infested soil or sand. Thirty to 40 seeds were sown per 6-in. pot, and each pot was thinned to 10–20 seedlings after all danger of damping-off was past. Sterile water was used in watering plants growing in soil, and 0.5*N* Hoagland solution was used for plants growing in sand. The 0.5*N* concentration was selected after preliminary tests with 0.25, 0.5, 1.0, 2.0, and 3.0*N* solutions.

Several soils and soil treatments were used. Most pure-culture studies were made in white silica sand or in a 3:1 greenhouse mixture of woods-soil and sand treated to eliminate pathogenic organisms. In some instances it was steamed 40 minutes at 10 lb pressure on 3 consecutive days; in other cases it was fumigated with propylene oxide by filling a 50-gal. steel drum with loose soil, punching 8–10 1-in. holes through the soil to the bottom of the container, introducing propylene oxide into the holes at the rate of 7 ml per gal. of soil, sealing the drum tightly, and leaving it 7 days at 30–38°C. The soil was then removed, placed in 6-in. sterile pots, and allowed to aerate for 7 days. Then the inoculum was added and the clover seeded.

Preliminary tests were made in soils from 5 different locations in Wisconsin: Miami silt loam from Madison, Almena silt loam from Marshfield, Omega loamy sand from Spooner, Plainfield sand from Hancock, and Ontonagon silty clay loam from Ashland. The Madison soil had a fairly long history of continuous red clover culture; the other soils had been cropped to oats, legumes, and corn in rotation except that the soil from Ashland had not grown corn for several years.

The fungi studied were isolated during a survey of red clover root rot in Wisconsin, and all are common in Wisconsin soils. Some of the isolates had been tested previously for pathogenicity on red clover seedlings (12) but not on older plants. In the preliminary screening trials, 49 isolates were tested. These included 8 isolates of *Fusarium oxysporum*, 9 of *F. solani*, 6 of *F. roseum*, 1 of *Rhizoctonia leguminicola* Gough & Elliott, 6 of *Rhizoctonia* spp., 6 of *Pythium* spp., 5 of *Gliocladium* spp., 3 of *Phoma* spp., and 5 of other less prevalent genera. On the basis of the results of the screening tests, 9 isolates were selected for further studies. These included 3 isolates of *F. oxysporum*, 3 of *F. solani*, 1 of *F. roseum*, 1 of *Rhizoctonia* sp., and 1 of *Gliocladium roseum* (Lk.) Bainier. Another isolate, *F. moniliforme*, previously obtained from red clover roots and added after the screening tests were

completed, was included in all subsequent experiments. Monoconidial isolates were used except for the *Rhizoctonia* isolate, which was propagated from a hyphal tip.

Cultures for inoculum were grown 10 days at room temperature on potato-cerelose agar in Petri dishes. Half the inoculum was incorporated into the soil or sand immediately before seeding; the other half was added when the plants were 6 weeks old. The amount used per 6-in. pot or ½-gal. crock on each occasion was the mycelial mats from 2 Petri dishes. For the first infestation, the mycelium plus adherent agar was ground to a paste in a blender, sterile water was added, and the mixture incorporated into the soil or sand. For the second infestation, the mycelial mats were cut into small pieces with a sterile scalpel and placed in small holes between the plants. When transplants were used, all of the inoculum was placed in the soil before transplanting.

The influence of temperature on the mycelial growth of the 10 isolates selected for further study was determined on potato-cerelose agar in Petri dishes, 20 ml of agar per dish, over a range of 4–36°C at 4° intervals.

Temperatures in the greenhouse tests ranged from 21 to 25°C unless otherwise specified. A few experiments were made to study the effects of temperature on disease development and on the response of red clover to clipping. In these tests, temperatures were (±1°) 16, 20, 24, and 28°. Air temperatures were the same as the soil temperatures in each case except that on sunny days they were slightly higher in the low-temperature houses.

Dry weights of inoculated and uninoculated plants were determined at the conclusion of the preliminary tests to help measure the effects of the isolates on plant development. These weights were obtained by cutting washed plants (roots and tops) into small pieces, placing them in metal cans, and heating them in an oven at 100°C for 24 hours or until they attained a constant weight.

Under conditions favorable for host development most of the isolates were only weakly pathogenic on post-seedling plants, i.e., root disease symptoms developed very slowly. Since growing conditions are not always favorable for red clover in the field and since root rots usually cause most damage when conditions are unfavorable, attempts were made to determine the effects of the fungi on weakened plants. Smith (20) has shown that carbohydrate reserves in roots are diminished during winter dormancy, and that both carbohydrate and nitrogen reserves are reduced by early-spring growth as well as by the initiation of new growth following each cutting. The fluctuations in root reserves are very great and sometimes reach a dangerously low level. Under such conditions, even weak pathogens would obviously have many opportunities to become established in clover roots, or, if already established, to increase their rate of development. Several methods of weakening the plants were investigated. One was to force plants into dormancy by low-temperature treatment until the reserves were depleted. This method was not used extensively, because exhausting the reserves took too much time. Instead, various clipping treatments were used. Clipping consisted of removing all leaves and petioles immediately above the crown. Age of plants when clipping began, as well as the number of clippings, was varied according to the purpose of the test. Smith and Graber (21) have demonstrated the effects of clipping treatments on root reserves.

In all pure-culture experiments, attempts were made to re-isolate from the clover roots the fungus used as inoculum. Isolations were also made from the roots of plants grown in the naturally infested soils. To minimize errors due to color changes all roots were washed and classified for disease severity as soon as possible after they were lifted. All experiments included at least 2 replicates and adequate checks.

Disease indices were based on surviving plants only; the number of dead plants in each test was recorded separately and was not included in calculating the disease indices. Nine severity classes (0–8) were used: 0 indicated no root discoloration; 8 indicated very severe root and crown rotting (plants nearly dead); and 2–7, intermediate levels of disease. Factors considered in determining disease indices were the amount of cortical rot in either the tap or secondary roots, and the amount of vascular discoloration in any part of the root system. The average severity rating for a sample was calculated by multiplying the number of plants in each class by the class value, adding these products, and dividing the sum by the total number of plants in all classes.

Experimental results.—*Preliminary pathogenicity tests.*—Forty-nine fungus isolates from diseased red clover roots were tested for pathogenicity on red clover in 2 preliminary trials. In the first test, the plants were not clipped and the effects of the fungi were recorded 20 days, 6 weeks, and 5 months after seeding. In the second trial, the foliage of the plants was clipped and removed at 10-day intervals for a 2-month period, beginning when the plants were 6 months old, and the data were recorded 2 months after the last clipping. Both tests were made in steamed soil in 6-in. clay pots in a greenhouse at 21–25°C. Forty seeds were sown per pot, and after the seedling data had been recorded the number of plants per pot was reduced to 20. Each test included 2 replicates.

1) *Experiment I.*—Many of the isolates caused seed-rotting, pre-emergence killing, and post-emergence damping-off, but none were very pathogenic on plants 3 or more weeks old. The data for 17 of the 49 isolates are given in Table 1; data for the other 32 isolates were similar. There was a wide range among isolates in pathogenicity on seedlings; isolate 6 of *Gliocladium roseum* killed all plants within 20 days, whereas isolate 100 of *Fusarium solani* had no effect on stand. Isolates of the same species varied almost as much as isolates of different species. Stands in pots infested with different isolates of *F. oxysporum* were 14–81% of the check. Stands in pots infested with different isolates of *F. solani* were 59–103% of the check. Most isolates of *Gliocladium roseum* were less

Table 1.—Relative pathogenicity of 17 fungus isolates on unclipped red clover, as indicated by stands 20 days after seeding and by disease indices and dry weights of the surviving plants 6 weeks and 5 months after seeding.[a]

Fungus and isolate no.	20 days after seeding	6 weeks after seeding		5 months after seeding	
	Stand (% of check[b])	Disease index[c]	Av. dry wt. per plant (mg)	Disease index	Av. dry wt. per plant (mg)
Check	100	0.0	152	0.0	7031
Fusarium oxysporum					
10	14	2.0	35	–[d]	–
17	74	1.0	168	3.0	3291
5	81	0.5	148	0.5	4277
Fusarium solani					
311	59	0.0	141	1.0	3982
25	81	1.0	108	1.0	3330
28	81	0.0	112	0.0	5656
22	81	0.0	146	2.0	2628
16	97	0.0	158	1.5	3155
100	103	1.0	188	2.0	3471
Rhizoctonia sp.					
19	22	0.0	113	–	–
Pythium spp.					
338	8	–	–	–	–
312	97	0.5	75	1.0	3962
Gliocladium roseum					
6	0	–	–	–	–
Trichoderma sp.					
70	52	1.0	87	1.0	4002
Rhizopus sp.					
94	74	0.0	139	0.0	4145
Unidentified spp.					
72	89	0.0	115	0.5	3067
344	89	0.5	88	0.5	5421

[a] Test made in steamed soil at 21–25°C.
[b] Stands in check pots averaged 90% of seeds sown.
[c] Disease index based on a scale of 0–8: 0 = no disease, and 8 = very severe.
[d] No data, because too few plants survived.

pathogenic than isolate 6 of this fungus.

To determine the pathogenicity of the isolates on plants surviving the seedling stage, half of the plants (1 replicate) were lifted when 6 weeks old, washed, and rated for external and internal root discoloration. As shown in Table 1, none of the plants showed much disease at this time. Only isolate 10 of *Fusarium oxysporum* had a disease index as high as 2.0, a low rating. To determine if the isolates had produced any less apparent effects, dry weights were determined for 15 of the inoculated lots of plants. Twelve of these had lower average dry weights than the check, suggesting a size reduction due to the fungi. The remaining plants (1 replicate) were harvested when 5 months old. Again there was little apparent root-rotting, though all inoculated lots had lower average weights than the check (Table 1). Several lots weighed less than half as much.

2) *Experiment II.*—Since none of the isolates were very pathogenic on vigorously growing unclipped post-seedling plants, attempts were made to determine their effects on plants weakened by clipping. The results are summarized in Table 2. Some isolates were still either nonpathogenic or only weakly pathogenic, but a few were moderately pathogenic, showing that these fungi can cause root rot of post-seedling red clover plants. The range in pathogenicity among the 49 isolates was 0–6.4. In all subsequent tests the number of isolates was reduced to 10.

Effects of temperature on mycelial growth of selected isolates.—The influence of temperature on the mycelial growth of the 10 isolates selected for further pathogenicity tests was determined on potato-cerelose agar (Table 3). Eight of the isolates produced most radial growth at 28°C; 2 isolates of *Fusarium solani* grew best at 32°. All the isolates grew over a wide range of temperatures. The slowest-growing isolate at optimum temperatures was *Gliocladium roseum;* fastest growing was *Rhizoctonia* sp. The most growth at 8° or less was produced by *Fusarium roseum;* most growth at 36° was produced by the 3 isolates of *F. solani.* Only isolate 254 of *F. roseum* produced measurable growth at 4° in 100 hours. All the isolates grew at 36°.

Effects of clipping foliage on the development of root rot.—Is was repeatedly demonstrated that clipping the foliage of red clover increased susceptibility of the plants to root rots. This was true regardless of whether the plants were grown in sand or soil, and regardless of age of plants at time of clipping. The amount of increase in susceptibility was influenced by the frequency of clipping, the age and vigor of the plants when clipping began, temperature, pathogen present, and other factors.

Table 2.—Relative pathogenicity of 49 fungus isolates on clipped red clover, as indicated by disease indices 10 months after seeding.[a]

Fungus and isolate	No. of plants	Disease index[b]	Fungus and isolate no.	No. of plants	Disease index
Check	68	0.0	*Gliocladium roseum*		
Fusarium oxysporum			6	9	5.8
5	10	6.4	228[c]	11	2.2
29[c]	11	4.7	176	14	1.6
17	6	3.3	7	3	1.3
10[c]	11	3.3	*Gliocladium* sp.		
132[c]	17	3.2	18	13	0.0
258	15	0.9	*Rhizoctonia leguminicola*		
12	16	0.7	134	3	1.3
251	12	0.0	*Rhizoctonia* spp.		
Fusarium solani			136	12	2.7
25[c]	8	4.7	250	10	2.4
28	11	3.3	24	16	1.9
106	9	3.1	23	15	1.3
300[c]	25	2.9	45[c]	15	0.3
100	12	2.8	19	2	0.0
16[c]	14	2.1	*Pythium* spp.		
311	13	2.0	312	13	1.9
51	10	1.8	208	12	1.1
22	11	0.6	338	12	0.8
Fusarium roseum			131	28	0.7
8	10	3.0	164	12	0.5
9	9	2.7	67	10	0.0
254[c]	14	2.0	*Trichoderma* spp.		
76	15	1.0	70	9	3.5
229	15	0.9	2	20	1.6
92	11	0.9	*Rhizopus* sp.		
Phoma spp.			94	5	2.9
212	10	0.6	Unidentified spp.		
257	12	0.3	72	18	5.3
221	9	0.0	344	13	1.5

[a] Test made in steamed soil at 21–25°C. Foliage of plants was clipped and removed at 10-day intervals for a 2-month period, beginning when the plants were 6 months old. Plants were lifted and the data recorded 2 months after the last clipping.

[b] Disease index based on a scale of 0–8: 0 = no disease, and 8 = very severe.

[c] Isolate selected for further tests.

Table 4 gives the results of one of the tests made in sand in ½-gal. crocks. It shows that clipped plants were 2–5 times as susceptible to most isolates as unclipped plants. Only the *Rhizoctonia* isolate used failed to incite more disease on clipped than unclipped plants. None of the isolates were more than weakly

Table 3.—Effects of temperature on radial growth of 10 fungus isolates on potato-cerelose agar in Petri dishes after 100 hours of incubation.[a]

Fungus and isolate	Diameter of colonies (mm) at indicated temperatures (°C)								
	4°	8°	12°	16°	20°	24°	28°	32°	36°
Fusarium oxysporum									
10	0.0	1.4	6.8	13.0	19.7	26.1	30.0	25.1	4.1
29	0.0	1.9	9.7	16.8	23.3	34.6	38.7	38.6	14.6
132	0.0	0.5	7.9	15.0	21.4	28.3	30.3	27.8	7.9
F. solani									
16	0.0	0.5	5.6	9.9	15.9	25.9	29.3	30.5	24.9
25	0.0	1.6	5.6	10.7	17.0	25.0	27.6	26.7	15.9
300	0.0	0.5	5.0	9.4	15.4	23.7	26.8	29.6	23.1
F. roseum									
254	1.0	5.0	8.8	12.5	14.9	15.2	16.3	8.6	3.6
F. moniliforme									
1	0.0	0.5	7.8	14.6	20.7	32.0	33.4	32.9	12.9
G. roseum									
228	0.0	0.5	4.0	7.2	10.8	15.8	15.9	15.3	6.8
Rhizoctonia sp.									
45	0.0	2.6	8.1	19.4	32.8	45.4	51.4	43.3	6.6

[a] Data are averages for 2 trials; 4 replicates in each trial.

pathogenic on unclipped plants but several isolates caused moderately severe rotting on clipped plants. The roots of the check plants in this test were remarkably free of discoloration in both the clipped and the unclipped series.

To determine the relation between frequency of clipping and susceptibility to root rots, plants—8 and 12 weeks old—were clipped at 3-day intervals for 2, 4, or 6 weeks. Unclipped plants were included in each age group for comparison. This test was made in soil fumigated with propylene oxide and artificially infested with the 10 isolates indicated in Table 4 immediately prior to seeding. The plants were removed from the soil for root examination 3 weeks after the last clipping. The experiment was a split-plot test with 2 replicates and 20 plants per treatment per replicate. The results showed that the effects of clipping plants 12 weeks old were greater than the effects of the same number of clippings of plants 8 weeks old, that the effects of clipping increased with the number of clippings, and that clipping at 3-day intervals for 6 continuous weeks was too severe regardless of the plant age. Ninety-three per cent of the check plants were killed by the 6-week clipping treatment and the survivors were in poor condition. Fig. 1 shows the effects of different amounts of clipping of 12-week-old check plants.

Table 4.—Effects of clipping foliage at 3-day intervals for a 12-day period, beginning when plants were 12 weeks old, on the susceptibility of red clover to 10 fungus isolates in sand.[a]

Fungus and isolate no.	Disease index[b]	
	Foliage not clipped	Foliage clipped
Check[c]		
Agar	0.0	0.0
No agar	0.0	0.0
Fusarium oxysporum		
10	1.0	5.0
132	1.0	3.3
29	0.9	3.0
Fusarium solani		
300	1.2	5.0
16	1.0	5.0
25	1.8	4.0
Fusarium roseum		
254	2.0	4.0
Fusarium moniliforme		
1	2.8	3.0
Gliocladium roseum		
228	0.8	3.8
Rhizoctonia sp.		
45	2.0	2.0

[a] Plants started from seed in steamed white silica sand artificially infested with the indicated isolates immediately before seeding. Data were recorded 3 weeks after last clipping.

[b] Disease index based on a scale of 0–8: 0 = no disease, and 8 = very severe. Dead plants were not considered in computing disease index.

[c] Two kinds of checks were used. In one, the same amount of sterile agar was added to the sand as was used to incorporate the inoculum in the infested series; in the other nothing was added.

Effects of temperature on the reaction of clipped and unclipped red clover to root diseases.—Since temperature influences rate of growth and, consequently, the rate of depletion of root reserves when plants are clipped frequently, an experiment was made to determine the relation of temperature to disease development in clipped and unclipped red clover. Seeding was done in steamed soil artificially infested with the 10 isolates in ½-gal. crocks in temperature tanks in each of 4 adjacent greenhouse compartments at 16, 20, 24, and 28°C. Half of the plants in each crock were clipped and the other half left unclipped. Clipping began when the plants were 12 weeks old. In half of the pots the plants were clipped only once; in the other half they were clipped at 3-day intervals for 12 days. The experiment included 2 replicates and each crock contained 15–20 plants when clipping began.

None of the unclipped plants or of those clipped only once were killed by any isolate at any temperature. Effects of clipping more than once increased as temperature increased. Relatively few plants were

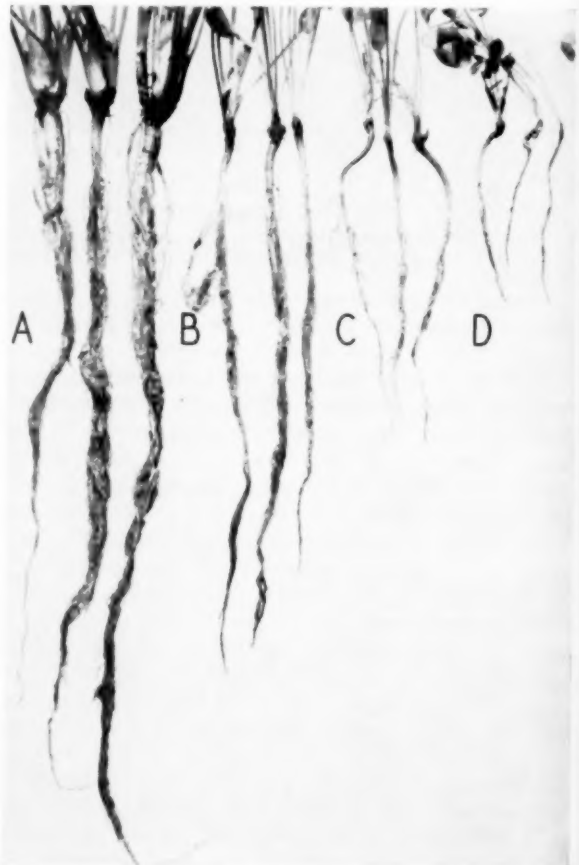

Fig. 1.—Effects of clipping foliage of healthy red clover plants at 3-day intervals for periods of 2, 4, and 6 weeks, beginning when the plants were 12 weeks old. Plants were grown in soil treated with propylene oxide, and no fungi were added to soil. **A)** Unclipped; **B)** clipped for 2 weeks; **C)** clipped for 4 weeks; **D)** clipped for 6 weeks. Photograph was made 3 weeks after last clipping.

Table 5.—Effects of soil type and temperature (°C) on disease severity and per cent plants killed in clipped and unclipped red clover grown in a greenhouse.[a]

Clipping treament and temperature	Disease index on plants grown in soil from[b]					% plants killed in soil from				
	Ashland	Hancock	Madison	Marshfield	Spooner	Ashland	Hancock	Madison	Marshfield	Spooner
Foliage not clipped										
16°	1.0	0.8	2.0	1.3	2.0	0	0	0	0	0
20°	3.5	2.0	1.0	3.0	1.0	0	0	0	0	0
24°	5.0	3.0	3.0	2.0	2.0	0	0	0	0	0
28°	6.0	3.8	2.0	3.8	4.0	0	0	0	0	0
Av.	3.9	2.4	2.0	2.5	2.3	0	0	0	0	0
Foliage clipped[c]										
16°	2.0	1.3	3.0	2.8	2.0	0	0	0	0	0
20°	5.0	2.0	2.3	7.0	7.0	60	20	20	25	50
24°	5.0	5.0	[d]	[d]	8.0	50	33	100	100	33
28°	8.0	[d]	8.0	8.0	8.0	75	100	85	50	75
Av.	5.0	4.1	5.3	6.4	6.2	46	38	51	44	40
Grand av.	4.4	3.2	3.6	4.4	4.2	23	19	26	22	20

[a] All soils naturally infested, with no fungi added. Healthy 8-week-old plants were transplanted to the various soils at the beginning of the experiment.

[b] Data based on a scale of 0–8: 0 = no disease, and 8 = very severe. Dead plants were not considered in computing disease index.

[c] Plants were 12 weeks old when clipping began, and were clipped 4 times at 3-day intervals. Plants were lifted and data recorded 3 weeks after last clipping.

[d] No surviving plants. In computing averages, a disease index of 8 was used.

killed at 16°C; nearly all plants were killed at 28°. Similarly, few of the isolates increased the amount of root rot in unclipped plants at any temperature. There was some browning of the secondary roots of all plants including those in the checks. Clipping the plants increased disease severity at all temperatures, and clipping 4 times had more effect than clipping only once. In general, disease increased with increases in temperature.

Effects of soil type on root rot development.—Soils from 5 locations in Wisconsin were used in greenhouse tests to determine their effects on root-rot development. Two kinds of experiments were made. In the first, the naturally infested soils were used as they came from the field, without steam or chemical treatment and without the addition of other fungi. In the second, the 10 isolates used in previous experiments were added separately to each of these soils to determine their effects in combination with the natural microflora. In both tests, healthy 8-week-old plants were transplanted to the various soils.

The first experiment was made in ½-gal. crocks in temperature tanks at 16, 20, 24, and 28°C. Half of the plants in each crock were clipped 4 times at 3-day intervals, beginning when the plants were 12 weeks old; the other half were left unclipped. The plants were lifted 3 weeks after the last clipping.

It was difficult to ascertain which soil microflora incited most disease (Table 5). Root-rotting occurred in all the soils. Among the unclipped plants, those grown in the Ashland soil had the highest disease index, particularly at the higher temperatures. There was little difference among the other soils. In the clipped series, most dead plants were found in the Madison and Ashland soils, but surviving plants grown in the Marshfield and Spooner soils had higher severity indices. Least disease occurred in the Hancock soil. In general, more disease developed at the higher temperatures (Fig. 2) and on clipped plants.

The second experiment was similar except that the plants were grown in 6-in. pots at a temperature of 21–25°C and the 10 isolates used in previous tests were added separately to each of the soils prior to transplanting. Adequate checks to which none of the isolates were added were included. Clipping treatments were the same as for the first experiment.

The results indicated little effect on unclipped plants of adding any of the 10 isolates to the naturally infested soils. No plants were killed and there was little if any increase or decrease in the disease severity index. When the plants were clipped, however, some isolates appeared to reduce disease severity in certain soils, some appeared to increase it, and still others had no apparent effect (Table 6).

Relative prevalence of fungi associated with root rots.—Isolations were made from diseased roots of red clover grown in naturally infested soils obtained from Ashland, Hancock, Madison, Marshfield, and Spooner. In general the results were the same as those reported by Kilpatrick et al (11). *Fusarium oxysporum* was the most prevalent fungus isolated from all 5 soils. *F. solani*, *F. roseum*, *Rhizoctonia* spp., and *Gliocladium* spp. were among other fungi commonly isolated. *Pythium* spp. were frequently obtained from seedlings but not from older plants. *Sclerotinia trifoliorum* was not isolated.

Discussion.—Although root and crown rots rank among the more important diseases of red clover, the fungi inciting them are, for the most part, weak pathogens. They cause little damage to vigorously growing plants but cause extensive damage to plants lacking in vigor. Under field conditions, plants become predisposed to attack in many ways. In the northern

areas, root reserves are largely consumed during the long winter dormancy so that the plants are less able to resist attack. In addition, winter injury to roots and crowns is frequent. In all clover areas, viruses, fungus pathogens, insects, excessive pasturing, drought, and low soil fertility predispose plants to attack. No field completely escapes damage from root and crown rots. The fact remains, however, that most of these fungi are weak pathogens on post-seedling plants, and the diseases they incite develop very slowly under conditions favorable for host development.

Age of plants affects susceptibility to root rots. Germinating seeds and small seedlings are very vulnerable to attack by several species of *Pythium* (7) and other fungi (13) that are less pathogenic on older plants. Plants surviving the seedling stage are much more resistant than they were earlier. Resistance to *Fusarium* spp. and certain other fungi, however, gradually breaks down if the plants become weakened.

Clipping of the foliage depletes root reserves and increases susceptibility to root rots. The more frequent the clippings the weaker and more susceptible plants become. Clipping is a useful technique for reducing the vigor of clover plants to the point where infection can be obtained and disease development accelerated. Since temperature, light, size and vigor of plant at time clipping begins, and other such factors greatly influence the response of plants to clipping, all these factors must be considered in determining how many times to clip to achieve a desired level of disease. Adequate checks are imperative in such tests to determine how much of the killing and root degeneration are caused by the fungus and how much are due to the clipping.

There are all degrees of damage from root rots. Damage varies from complete destruction of the root system and killing of the plants to no apparent effects. Both primary and secondary roots may be invaded. Decay may be mostly cortical, mostly vascular, or both cortical and vascular. Secondary roots of field-grown plants are almost invariably infected and discolored. The intensity of discoloration is usually indicative of the amount of damage: the darker the roots the greater the damage. Under field conditions, fungi continually prune away small or large areas of the root system. Such losses reduce the productivity of plants as well as render them more susceptible to further attacks by root pathogens. There is also some evidence that plants grown in close association with root-rotting fungi are stunted as indicated by reduced dry weight,

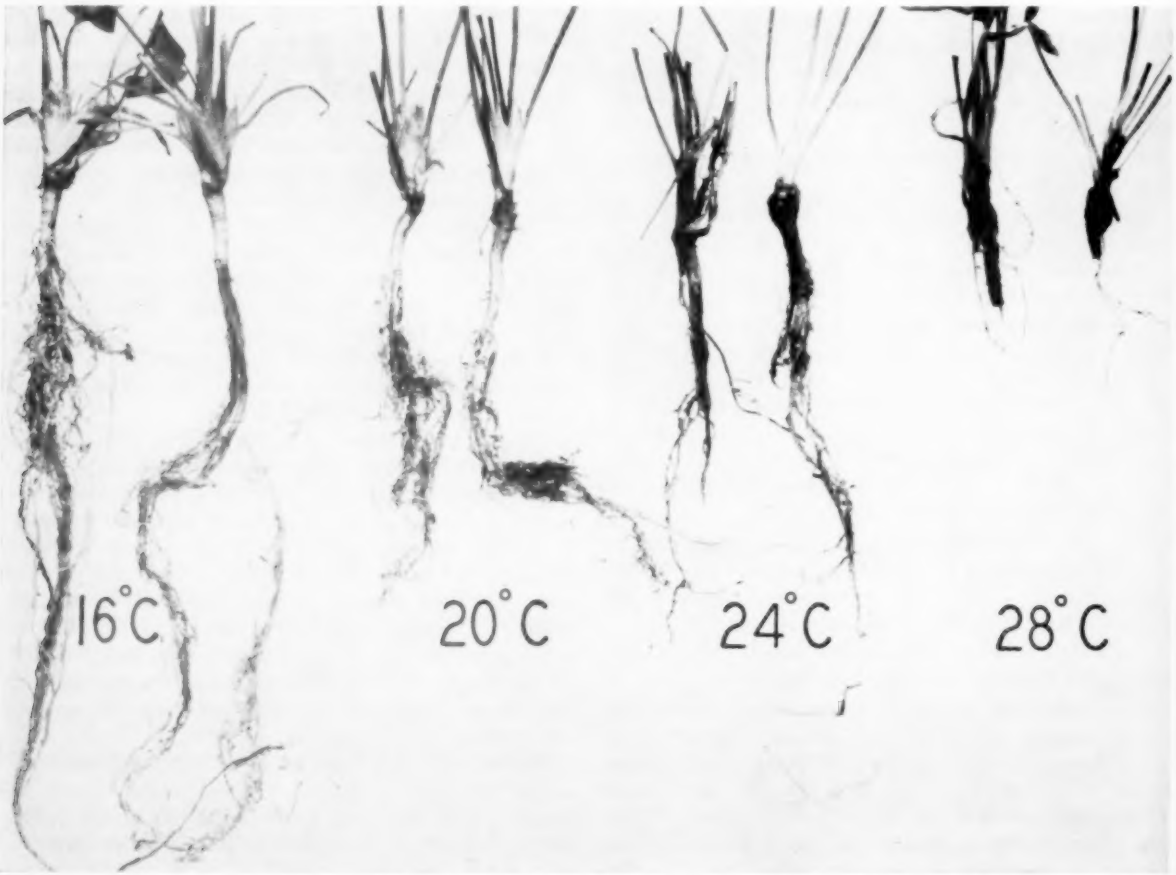

Fig. 2.—Effects of temperature on the development of root rot of red clover in naturally infested soil from Ashland, Wisconsin. Healthy 8-week-old plants were transplanted to the soil and the foliage clipped 4 times at 3-day intervals, beginning when the plants were 12 weeks old. Photograph was made 3 weeks after the last clipping.

Table 6.—Effects of 10 fungus isolates on clipped red clover plants grown in the greenhouse at 21–25°C in naturally infested soils from 5 location in Wisconsin.[a]

Fungus and isolate no.	Disease index on plants grown in soil from[b]					% plants killed in soil from				
	Ashland	Hancock	Madison	Marsh-field	Spooner	Ashland	Hancock	Madison	Marsh-field	Spooner
Check[c]										
Agar	7.0	4.5	5.5	4.0	4.0	80	33	76	57	50
No agar	[d]	[d]	6.0	6.0	3.0	100	100	58	60	40
F. oxysporum										
10	3.0	4.0	5.0	[d]	7.0	0	80	46	100	17
29	3.7	6.0	7.0	3.0	4.0	17	83	62	43	20
132	4.0	4.0	6.5	3.0	4.0	80	33	44	0	40
F. solani										
16	[d]	4.7	4.0	3.0	4.0	100	40	53	57	0
25	3.0	4.0	5.5	3.0	5.0	83	20	24	14	20
300	[d]	6.0	5.0	3.0	5.0	100	33	36	33	33
F. roseum										
254	4.0	4.5	6.0	4.0	4.0	33	29	36	29	57
F. moniliforme										
1	4.0	4.0	5.0	3.0	4.0	25	83	42	11	17
G. roseum										
228	4.0	6.0	5.0	3.0	4.0	57	60	52	14	57
Rhizoctonia sp.										
45	4.0	4.0	4.5	3.5	4.0	60	50	63	0	83
Av. all isolates and checks	5.1	5.0	5.4	3.9	4.3	61	54	49	35	36

[a] Plants were transplanted to indicated soils when 8 weeks old, and lifted for root examination when 17 weeks old. Clipping of foliage was begun 4 weeks after transplanting and continued at 3-day intervals for 12 days.

[b] Data based on scale of 0–8; 0 = no disease, and 8 = very severe. Dead plants were not considered in computing disease index.

[c] Check = naturally infested soil with no fungus added. Two kinds of checks were used. In one, the same amount of sterile agar was added to the soil as was used to incorporate the inoculum in artificially infested pots; in the other nothing was added.

[d] No surviving plants. In computing averages a disease index of 8 was used.

even when the roots show no symptoms.

Most of the fungus isolates studied grew best at 28–32°C, and it appeared that root rots were generally more severe at high temperatures. These fungi, however, grew over a wide temperature range and caused damage at temperatures considerably below their optimum for growth.

All the naturally infested field soils contained root-rotting fungi. Plating tests showed that the microflora of red clover roots grown in these soils were similar for all the soils though not identical. Differences in the relative severity of disease in the various soils were not consistent over all conditions, but least disease occurred in the Hancock soil. When pure cultures of various fungi were added to these soils, disease severity was reduced slightly in most cases. Much remains to be done on the effects of the various fungi on each other and on disease development.

Since the problem is complex, its solution is dependent on the development of suitable methods and techniques. Since many factors are involved, the writers feel that the most promising approach is first to study each factor separately, holding all others as constant as possible. When this has been done the various factors can be studied in combination to determine their interactions.

The best method of controlling root rots is to keep the plants growing vigorously. This cannot be done at all times, but sturdy adapted varieties, suitable liming and fertilization, proper crop rotation, and good management should help. In attempting to develop resistance to this disease complex, first consideration must be given to selecting or developing plants with superior inherent vigor and the ability to tolerate unfavorable conditions.—Department of Plant Pathology, University of Wisconsin, Madison.

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RELATIVE PATHOGENICITY OF TWO BROWN ROT FUNGI, SCLEROTINIA LAXA AND SCLEROTINIA FRUCTICOLA, ON TWIGS AND BLOSSOMS

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SUMMARY

Cankers produced by *Sclerotina laxa* and *S. fructicola* on Drake and Mission (Texas) almond, Royal apricot, Quetta nectarine, Italian prune, and Walton peach showed, in general, a similar reaction to seasonal conditions. *S. laxa* produced longer cankers than *S. fructicola*. Air temperature and canker length were correlated. Gumming produced on the inoculated area apparently was related not to host resistance but to inoculation when the cambium was active. Typical isolates on PDA produced similar cankers. Cankers were longest on almond, followed in order by apricot, peach, and nectarine. Seven days after inoculation of Drake almond, cankers produced by *S. laxa* and *S. fructicola* were respectively 114 mm and 71 mm long.

Blossom inoculations showed no distinct differences in pathogenicity between *S. laxa* and *S. fructicola*, though the latter generally produced somewhat more blossom blight. Closed blossoms were less susceptible to blossom blight than open blossoms. Separate inoculation of various floral parts showed that the stigma was most susceptible to infection and, when infected, led to the greatest amount of blossom blight, especially when the protruding stigma of closed blossoms was inoculated. Inoculations inside the floral tube gave percentages of blossom blight almost equal to those from stigma inoculations. The likelihood of inducing blossom blight by inoculating other floral parts was greatest for anthers, followed by filaments, petals of unopened blossoms, floral tubes of closed blossoms, and petals of open blossoms.

The blossoms of peaches and almonds were markedly less susceptible to infection before than after opening, whereas those of plums and apricots showed little or no difference in susceptibility with openness.

Leachates from the petals and floral tubes of closed blossoms and the petals of open blossoms increased spore germination and germ-tube growth. No distinct differences in reaction existed between the two brown-rot pathogens. In almost every test, spore germination and germ-tube length were decidedly greater in water drops exposed to stigmas, anthers, or the insides of floral tubes of open blossoms than in water drops exposed to other floral parts. No differences in leachate stimuli from blossom parts were found among the various hosts tested. Pollen-laden floral parts were more prone to infection by both *S. laxa* and *S. fructicola*.

Field inoculations made with mycelium yielded low percentages of blossom blight when the stigma was inoculated, and none when other floral parts were inoculated. The technique appeared unsuitable for appraisal of blossom susceptibility in the various hosts. In a single test, made on Bing cherry in 1953, inoculation of the stigma with dry spores yielded more blossom blight than did inoculation with mycelium on the stigma of most other hosts. The indications are that, in the production of blossom blight in the field, dry spores or mycelium give fairly good results if applied on the stigmas, but not on the other floral parts. The moisture and nutrients on the stigma should favor fungus growth.

Though all commercial species of *Prunus* are attacked by *Sclerotinia fructicola* (Wint.) Rehm [= *Monilinia fructicola* (Wint.) Honey] and *S. laxa* Aderh. & Ruhl. [*M. laxa* (Aderh. & Ruhl.) Honey], certain species are more often attacked by one fungus than the other. In California, for example, the brown-rot diseases of peach (*P. persica* Batsch.) and almond fruit (*P. amygdalus* Batsch.) are more often caused by *S. fructicola* than by *S. laxa*. The brown-rot blossom-blight disease of almond and apricot (*P. armeniaca* L.), on the other hand, is largely caused by *S. laxa*.

In California, moreover, the two fungi exhibit marked differences in their infection of the various parts of the hosts (19). *S. fructicola* infects peach blossoms but seldom causes the extensive blighting of blossoms and twigs produced by *S. laxa* on almond and apricot (9, 11, 21). Likewise, *S. laxa* attacks ripe apricot fruit and occasionally green almond fruit, but it seldom causes extensive fruit rot like that produced by *S. fructicola* on peach.

Elsewhere, the host and host-part preferences of the two fungi are exhibited to a lesser degree. *S. laxa* causes extensive damage to blossoms and twigs of sour cherry, apple, and plum (28, 29, 30) in Europe and to sour cherry in Wisconsin (2). *S. fructicola* causes extensive damage to peach blossoms in eastern and northwestern United States (1, 20).

Inoculations of twigs have been made successfully with *S. laxa* (12 and *S. fructicola* (6, 10, 13, 26). Chester (4), in 1893, was probably the first to produce blossom blight of peach by artificial inoculations. Detached blossoms of stone-fruit species have since been artificially inoculated with *S. laxa* (2, 29) and *S. fructicola* (20, 25) and natural blossoms in the orchard have been successfully inoculated with *S. laxa* (8, 27) and *S. fructicola* (12, 15).

Studies were made to compare the pathogenicity of the two organisms on various *Prunus* hosts by artificial inoculations of twigs and blossoms (17).

Materials and methods.—Single typical species of *S. laxa* from apricot and *S. fructicola* from peach, as determined by the method of Hewitt and Leach (9), were the test organisms. The description and behavior of these isolates in comparison with other isolates of both pathogens collected in California have been reported (18). *Prunus* species used in these studies are in the University of California variety plot at the Wolfskill Experimental Station, Winters, California.

Results and discussion.—*Twig inoculations.*—a) *Canker development in different seasons of the year.*—Inoculations on Royal apricot, Quetta nectarine, and Drake almond were made at 2–4-week intervals for a period of one year, and Walton peach, Texas almond, and Italian prune were inoculated at similar intervals for a period of 4 months. On each host, 10 inoculations were made on one-year-old twigs with each of the two fungi, and 10 checks with sterile PDA (1.5% potato-dextrose agar). The bark was removed with a 4-mm cork borer, a 4-mm disc was cut from the outer edge of two-day-old PDA plate cultures, and the mycelium-bearing side was placed at the cambial layer. The bark was then replaced and the inoculation wound wrapped with masking tape to reduce desiccation. The

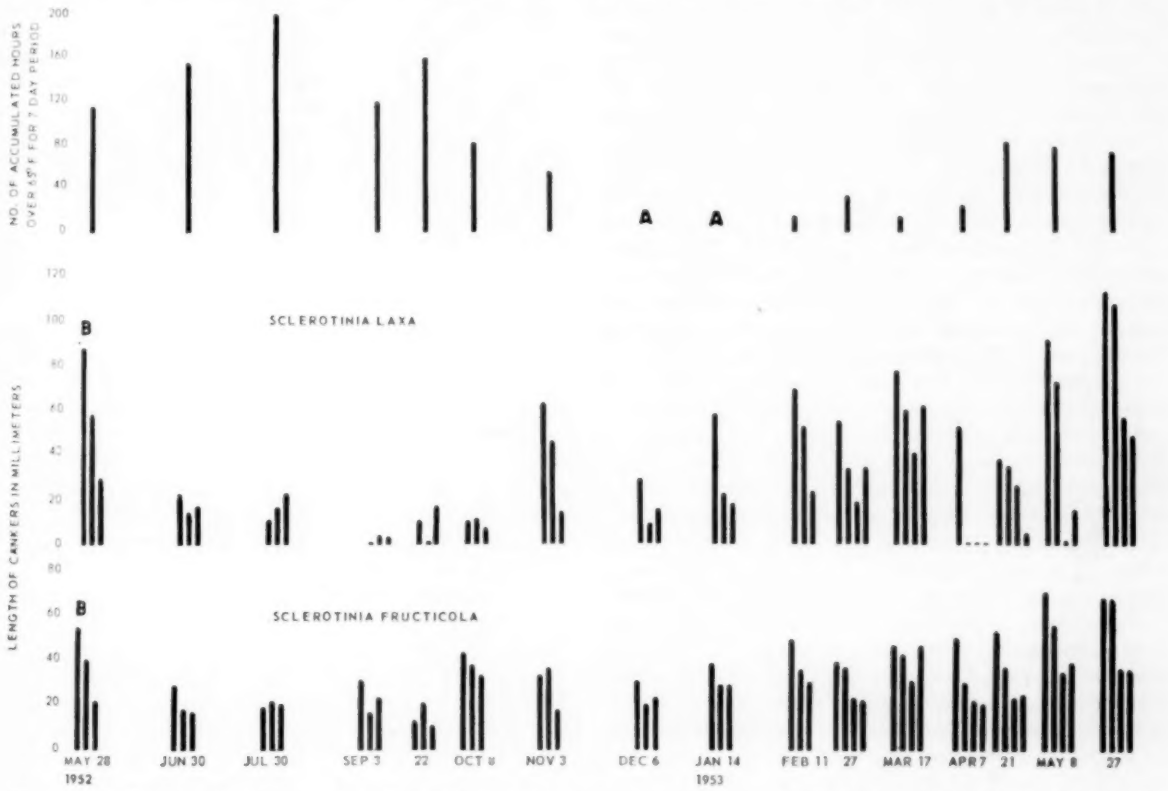

Fig. 1.—Canker length pattern is similar with both fungus species. Each produces the longest cankers in late spring. Almond inoculations gave largest cankers, followed by apricot, and then by peach and nectarine, which were approximately the same. Smallest cankers were produced during the hot summer and cold winter months. **A)** Temperatures did not rise over 65°F. **B)** Each inoculation group represents, left to right, Drake almond, Royal apricot, Quetta nectarine, and Walton peach. Peach inoculations were made only from February 27 to May 27, 1953.

tape was removed 7 days later, when readings were made on canker length. In the final computations, the diameter (4 mm) of the inoculation point was subtracted from the total length of each canker.

Inoculations during a two-year period (Fig. 1) showed that the general trend in canker size in Drake almond, Royal apricot, Quetta nectarine, and Walton peach with both organisms appears to be a gradual increase from winter to spring. Cankers were formed most rapidly during the late spring; activity was least in the summer and slight in the fall. In general the cankers produced by the two brown-rot species were similar, but there appeared to be a tendency of *S. laxa* to produce longer cankers than *S. fructicola* during winter and spring months.

When average canker lengths in 12 inoculations with *S. laxa* and *S. fructicola* on almond, nectarine, and apricot were analyzed statistically, there were no significant differences between the organisms, but differences existed between hosts. Mean canker lengths in mm of *S. laxa* and *S. fructicola* were 42.6 on almond, 34.3 on apricot, and 24.5 on nectarine. Differences were significant at the 1% level between almond and nectarine, and nectarine and apricot, and at the 5% level between almond and apricot. In general, the inoculations of almond produced the longest cankers, followed by apricot and nectarine.

b) *Canker size related to temperature and season.*—The number of hours over 65°F from the time of inoculation to the time of reading was calculated from a continuous temperature chart (bar graph in Fig. 1). (The charts from which the data were taken were obtained from Professor Dillon S. Brown, Pomology Department, University of California, Davis.) Fairly good correlation was shown between temperature and canker size. Cankers were shortest from inoculations made in the summer, and longest from inoculations made in the spring or fall. That small cankers are formed during the winter may be the result of cold weather on both host and pathogen, the host being dormant and lacking actively growing tissues.

c) *Gumming not an indication of resistance.*—A heavy flow of gum has indicated greater resistance in sweet and sour cherries inoculated with conidia of *S. cinerea* (*S. laxa*) (5, 16, 22). On apricots Vielwerth and Slink-Mezencovova (24) concluded that the infective capacity of the fungus was related to the rate of healing of the injured tissues. In the present studies gumming from pathogen-inoculated twigs was directly related to the presence of callusing in the checks. Cankers were longest when inoculations were made during the period when the bark could be removed most easily. Inoculations in highly susceptible Drake almond and resistant Mission varieties with either brown-rot pathogen produced no significant differences in gumming or canker length. Although Huber and Baur (12) could not infect Italian prune with either pathogen, inoculations of this variety made on May 27, 1953, produced cankers 43.3 mm and 37.7 mm long, respectively, for *S. laxa* and *S. fructicola*, with gumming at the inoculation point. Inoculation of Mission variety of olive with both pathogens on April 7, 1953, failed to produce extensive necrosis, and no gumming was observed.

d) *Size of mycelial mass determines canker length.*—Fungus mycelium or spores have been used in inoculating wounded shoots (3, 6, 9, 12, 13, 26). Cankers in these tests have shown great variability in length.

Inoculum consisting of mycelium growing on a disc of 1.5% PDA caused larger cankers than did inoculation with a water suspension of conidia. The mean length was 69.1 mm for cankers produced by *S. laxa* from mycelial disc inoculation on Drake almond in February, 1953, and 47.0 mm for *S. fructicola*, whereas inoculations with a spore suspension produced cankers of mean lengths of 17.6 mm and 6.3 mm, respectively. Judging from this, nutrients in the inoculum may increase canker size. To determine if different nutrients in the agar discs used in inoculation influence canker size, five media were compared. Potato extract added to the agar medium increased canker size, but dextrose and asparagine added to the potato agar resulted in no consistent increase. Since only water agar produced sparse mycelial growth, it appears probable that the longer cankers associated with the more nutritious media resulted mainly from the greater amount of mycelium introduced in the inoculum.

e) *Location of fungus in canker tissues.*—Isolations made from the tissues in different parts of the bark cankers and from points beyond the canker margin showed that the mycelium was present at the margins of the visible canker. Longitudinal sections could not verify the presence of mycelium in the advancing margins of the necrotic tissue. Since cultures had revealed the presence of the fungus in such tissues, the hyphae must be sparse or difficult to see. Well defined periderm formation was observed well in advance of the necrotic area, which indicates that the limiting factor in canker size may be the effect of the host on the pathogen.

f) *Inoculation date influences the period of canker enlargement.*—To determine if cankers caused by *S. laxa* and *S. fructicola* may continue to enlarge after one week, cankers on almond, apricot, and nectarine from inoculations of June 30, 1952, were outlined with a wax pencil on July 7 and remeasured on July 17. There were no measurable increases in canker size. All cankers from inoculations made from May 28 to December 6, 1952, were remeasured on January 24, 1953. Cankers from both pathogens increased in size after one-week incubation period on these three hosts except for those which formed callus tissues at the advancing margins of necrosis. With almond, callus formed at the inoculation point in almost all inoculations from May 28 to September 22; with apricot and nectarine, callus formed in inoculations made between May 28 and September 3. Increases in canker length (Table 1) were pronounced in inoculations made on October 8 and December 6 on almond, from September 22 to December 6 on apricot (except the September 22 inoculations with *S. laxa*, which failed to en-

Table 1.—Increases in canker length after 7 days past inoculation (inoculations from May 10 to September 3 failed to make significant increases in canker length on the three hosts).

Date of inoculation (1952)	Increases in canker length (mm)					
	Almond		Apricot		Nectarine	
	Sl [a]	Sf [a]	Sl	Sf	Sl	Sf
September 22	0	0	0	11.8	5.0	3.1
October 8	1.5	13.7	10.9	12.0	4.3	4.0
November 3	0	3.3	37.2	16.5	18.0	9.3
December 6	8.2	11.0	18.9	17.0	19.8	24.4

[a] Sl = *Sclerotinia laxa*; Sf = *S. fructicola*.

large), and from September 22 to December 6 on nectarine.

g) *Pathogenicity varies with isolates.*—All the previous inoculations were made with a single typical isolate of each species, so a study was made to determine whether canker size would differ with other isolates.

Inoculations were made on a single Drake almond tree with five different isolates of both species. The cankers were measured 7 days later. The stock culture of *S. laxa* (from apricot) produced cankers significantly smaller than those produced by a culture from almond, though considerably longer than those produced by the two atypical isolates from plum and apricot. The lesions caused by the *S. fructicola* stock culture (from peach) differed from those caused by the two atypical nectarine and peach cultures. Cankers produced by the two typical *S. fructicola* isolates from cherry and peach were not significantly different in size.

The results indicate that different isolates of *S. laxa* and *S. fructicola* may show marked variation in their ability to produce twig cankers on Drake almond. Culturally typical isolates (on PDA) of these two species, however, show relatively slight differences in size of cankers produced.

Blossom inoculations in the laboratory—procedure.—Blossom inoculations were made on the following hosts: Drake and Nonpareil almond, Royal, Smyrna, Hersey Moorpark, and Tilton apricot; Santa Rosa and Emilie plum; Stuart, Halford No. 2, Vedette, and Oriole peach; Napoleon and Bing cherry; and Winter Banana apple.

Blossoms used in the inoculation experiments were cut at the desired stage in the field. Unless otherwise specified, the inoculations were made at two stages of blossom development: a) closed, when the petals protruded beyond the calyx but the inner floral parts were not visible, and b) open, when the blossoms were at the full-bloom stage, with the inner floral parts well exposed and the pollen sacs intact. Upon collection, the shoots with the blossoms were immediately placed in a beaker of water. The stems of the shoots were recut under water and the shoots were divided into groups providing 60 blossoms for each type of inoculation.

In the closed flowers inoculations were made on sepals, petals, and the abaxial surface of the floral tube. In the open blossoms, the stigma, closed anthers, filaments, adaxial surface of the petals, and the adaxial and abaxial surfaces of the floral tube were inoculated. The spores of *S. laxa* and *S. fructicola* had been produced on Emperor grapes (2) that had been surface-sterilized for 20 minutes with a 1:9 solution of sodium hypochlorite and water.

In 1951, several techniques were used in developing a feasible method of floral infection. In 1952, spore suspensions were applied to the blossoms with an L-shaped nichrome wire, holding about 500 spores, and by atomizing a spore suspension on the blossom. In 1953, inoculations were made with a camel's-hair brush of a few strands. The spores were in a water-paste, except that inoculations inside the floral tube were made with droplets of spore suspension. Immediately after inoculation of the blossom parts, the floral clusters were incubated for 24 hours in a saturated-atmosphere chamber at 20±1°C and then stored 5 days at 22°C in a second chamber. The 20° chamber was built on the principle described by Keitt and Jones (14). A synthetic material, Dacron, sewed together with Nylon thread, was found to withstand the attacks by microorganisms better than muslin cloth. The inner chamber was maintained at 20±1°C and 100% RH.

The 22°C chamber consisted of a redwood frame, 20 in. wide, 40 in. long, and 18 in. high, covered with Plastiglass to permit light to enter. Humidity was maintained at about 82±2% RH with a saturated solution of potassium bromide. The temperature was 22±2°C (23). A squirrel-cage fan inside the chamber provided 6 mph air movement 12 in. from the outlet of the fan.

Blossoms were classified as blighted when necrosis had extended into the peduncle. From each test lot, 10 necrotic pedicels were surface-sterilized with sodium hypochlorite (1:9 dilution) for 2 minutes and plated on pea-straw water agar (7), a special medium favoring rapid growth and sporulation of the brown-rot organisms. The commonly isolated contaminants were *Penicillium* spp., *Sclerotinia sclerotiorum*, *Alternaria* spp., *Rhizopus* spp., *Aspergillus* spp. and *Botrytis* spp.

S. fructicola more pathogenic to blossoms than S. laxa.—In 1953 inoculations were made with both brown-rot organisms on open and closed blossoms of almond, apricot, plum, peach, cherry and apple. The results (Table 2) indicate that, in each host, *S. fructi-*

Table 2.—Relative susceptibility to blossom blight of 5 stone fruits and apple.

Host	No. varieties	No. blossoms used for each test	% blossom blight	
			S. laxa	*S. fructicola*
Almond	2	720	23	32
Apricot	3	1020	61	76
Plum	3	1020	48	67
Peach	4	1260	7	33
Cherry	2	540	86	93
Apple	1	180	13	16

Table 3.—Order of susceptibility of different floral parts from inoculations with *Sclerotinia laxa* and *S. fructicola* as expressed in per cent blossoms infected and blighted.

Host and variety	Organism[a]	Petals in closed blossom		Closed floral tube		Stigma		Anther		Filament		Open petal		Inside open floral tube	
		inf.[b]	bl.[b]	inf.	bl.	inf.	bl.	inf.	bl.	inf.	bl.	inf.	bl.	inf.	bl.
Almond															
Drake	Sl	100	12	0	0	100	48	28	8	8	3	dh[c]	dh	38	35
	Sf	100	8	10	2	100	100	100	50	47	23	dh	dh	78	78
Nonpareil	Sl	52	0	0	0	98	58	67	25	7	3	dh	dh	76	48
	Sf	15	0	0	0	92	35	12	5	3	3	dh	dh	78	78
Apricot															
Royal	Sl	100	35	0	0		57		63		70		42		97
	Sf	100	85	93	42		80		98		92		52		100
Smyrna	Sl	100	100	55	27	100	98	100	100	100	98		27		
	Sf	98	98	55	53	100	100	100	100	95	92		55		
Tilton	Sl	98	60	15	15										
	Sf	98	93	10	10										
Hersey Moorpark	Sl		75		68										
	Sf		100		48										
Plum															
Santa Rosa	Sl	100	23	18	18	93	27		34	12	5	100	0	39	39
	Sf	100	44	27	27	100	100		40		47	100	2	86	86
Emilie	Sl	90	81	7	5	100	100	100	95	98	83	93	34	100	97
	Sf	100	97	22	18	100	100	88	88	98	98	100	25	100	100
Cranberry	Sl		83		12										
	Sf		100		42										
Peach															
Stuart	Sl	0	0	0	0	20	2	10	5	3	2	0	0		
	Sf	100	0	34	3	93	62	100	53	100	35	73	5		
Halford #2	Sl	100	0	3	2	68	34	72	8	13	5	100	3		
	Sf	100	3	23	5	83	73	100	97	100	42	100	3		
Vedette	Sl	100	0	8	2	48	34	100	5	83	3	60	8		
	Sf	100	27	20	7	72	72	86	80	93	44	58	39		
Oriole	Sl		8		2										
	Sf		12		3										
Cherry															
Napoleon	Sl	97	97	48	27	100	100	97	81	100	85	100	83	100	100
	Sf	95	95	95	90	100	100	100	100	100	100	97	93	100	98
Bing	Sl	90	90	28	22										
	Sf	97	97	75	63										
Apple															
Unknown	Sl						20		10				10		
	Sf						8		37				2		

[a] Sl = *S. laxa*; Sf = *S. fructicola*.
[b] inf. = per cent blossom infection; bl. = per cent blight.
[c] dh = dehisced.

cola produced somewhat more blossom blight than did *S. laxa*, that peach was more resistant to *S. laxa* than the other fruit species, and that apple was somewhat susceptible to both fungi.

Susceptibility of floral parts of various hosts.—The relative susceptibility of the various floral parts of each host to the two fungi was indicated by the percentages of blossom blight resulting from separate inoculations of petals and floral tubes of closed flowers and stigmas, anthers, filaments, petals, and floral tubes of open flowers (Table 3). Infection was determined by water-soaked appearance or necrosis and surface isolation of the pathogen from such areas.

a) *Almond.*—In closed blossoms, Drake appeared more prone than Nonpareil to both infection of floral parts and resultant blossom blight. In the open bloom, inoculations of the stigmas, anthers, and filaments with *S. fructicola* produced more blight in Drake than in Nonpareil. With stigma and anther inoculations of Nonpareil, *S. laxa* produced more blight than did *S. fructicola*, but with filament inoculations the two fungi caused similar amounts of blight. The two varieties inoculated inside the floral tube showed no differences in percentage blight with *S. fructicola*, but with *S. laxa* the Nonpareil variety was somewhat more prone to infection and blight.

b) *Apricot.*—In inoculations of closed blossoms, floral tube inoculation produced less blossom blight than petal inoculation. At this stage of bloom Smyrna and Hersey Moorpark were more susceptible to blossom blight than Tilton and Royal. In the open-blossom stage also, Smyrna is more susceptible to blossom blight than Royal.

c) *Plum.*—Inoculations of petals at the closed stage

Table 4.—Comparison of percentage blossom blight in 5 stone fruits resulting from infection of closed and open blossoms with *Sclerotinia laxa* and *S. fructicola*.

Host	Number of varieties	Per cent blossom blight			
		S. laxa		*S. fructicola*	
		Closed	Open	Closed	Open
Peach	3	4	9	11	50
Almond	2	3	33	3	47
Plum	2	44	51	65	69
Apricot	2	48	54	66	85
Cherry	1	59	90	84	98

produced much blight of Emilie and Cranberry plum blossoms, but not of Santa Rosa plum. Open floral tube inoculations gave higher percentages of blossom blight in Emilie plum than in Santa Rosa plum. Inoculations of petals at the closed stage showed the order of decreasing susceptibility to be Cranberry, Emilie, and Santa Rosa. However, the closed floral tubes of Emilie appeared to be somewhat more resistant to blight than those of the other two varieties. With inoculations of open blossoms, Emilie plum was definitely more susceptible to blossom blight than was Santa Rosa plum.

d) *Peach.*—Inoculation of closed blossoms gave considerably less blight than did inoculation of open blossoms. In general, stigma and anther inoculations produced the highest percentages of blossom blight. Petal inoculation gave a high incidence of infection, but low percentages of blossom blight, apparently because the fungus failed to spread beyond the petals, perhaps because of dehiscence of the petals. In Oriole peach, inoculation of stigmas protruding beyond the petals of closed blossoms produced more blossom blight than did inoculation of the floral tube or petals. In Stuart and Halford No. 2 peaches, the percentage of blossom blight resulting from inoculation at the unopened stage was too low for a comparison of susceptibility. In petal inoculations of closed blossoms with *S. fructicola*, Vedette peaches developed the most blossom blight, followed by Oriole, Halford No. 2, and Stuart, in that order. Inoculation of the open blossoms of Stuart, Halford No. 2, and Vedette with either fungus failed to show any differences in varietal susceptibility to blight, except that the *S. laxa* inoculations of the stigmas of Stuart developed only 2% blight, compared with 34% in the other two varieties. Of inoculated floral parts, anthers and stigmas gave the highest percentage of blossom blight, and petals and floral tubes the lowest.

e) *Cherry.*—On both varieties of cherries studied, both organisms produced high percentages of blight, with no sharp differences in pathogenicity except in the closed floral tube inoculations. The varieties did not differ appreciably in their susceptibility to the two fungi. Of the Napoleon checks 3% of the closed blossoms and 5% of the open blossoms were blighted with *S. fructicola*. Many of the checks were blighted by contaminants, primarily *Botrytis* and *Sclerotinia sclerotiorum*.

f) *Apple.*—Inoculations of the blossoms of Winter Banana apple showed no sharp differences in the percentage of blight produced by the two brown-rot organisms. The percentage of blossom blight was comparatively low, the highest being 37%. With *S. laxa*, the highest percentage of blossom blight was from stigma inoculation, whereas with *S. fructicola* it was from anther inoculation.

g) *Open blossoms more susceptible to blight than closed blossoms.*—The blossom blight resulting in 5 stone fruits from infection of closed and open blossoms by two brown-rot organisms was compared (Table 4). The results show that peaches and almonds were markedly less susceptible to infection before the blossoms open, that cherries were slightly less susceptible, and that plums and apricots showed little or no difference. Blossom blight was less in peaches and almonds than in cherry, and plums and apricots were rated in between, with an average of about 60% blossom blight. *S. fructicola* produced more blossom blight than *S. laxa* in all cases except unopened blossoms of almonds, where blossom blight by each organism was only 3%.

h) *Stigmas most susceptible to blight.*—In general the two brown-rot organisms were similar in their capacity to infect the various floral parts (Table 5). Stigma and anther inoculations were most important except with *S. fructicola* on plum anther. Also highly important were petal infection in cherry and filament infection in apricot. The various floral parts were

Table 5.—Order of susceptibility to blossom blight from the inoculation of different floral parts with *Sclerotinia laxa* and *S. fructicola*.

Host	Number of varieties	Closed				Open							
		Petal		Floral tube		Stigma		Anther		Filament		Petal	
		Sl	Sf	Sl	Sf	Sl	Sf	Sl	Sf	Sl	Sf	Sl	Sf
Almond	2	4[a]	4	5	5	1	1	2	2	3	3	6	6
Apricot	2	4	3	6	6	3	3	1	1	1[b]	1	5	5
Plum	2	2	2	5	5	1	1	1	4	4	2	6	6
Peach	3	4	5	4	6	1	2	2	1	2	3	4	4
Cherry	1	1	1	6	1	1	1	3	1	3	1	3	1
Order of general susceptibility summarized		4		6		1		2		3		5	

[a] 1 indicates the highest percentage of blight, 6 the lowest. Where the ratings for *S. laxa* (Sl) and *S. fructicola* (Sf) are the same, the difference between the percentages of blossom blight is less than 10%.

[b] If the difference between floral parts in total blossom blight was less than 10%, the same rating is given and the consecutive numerical rating system is not used, e.g., the rating of 2 is omitted on apricots.

rated in susceptibility to blossom blight in decreasing order as follows: Stigma, anther, filament, closed blossom petal, open blossom petal, and floral tube.

The protruding stigmas of closed blossoms of Cranberry plum and Oriole peach (Fig. 2) were also inoculated. C. O. Hesse (University of California, Davis) has noticed that warm winters favor stigma protrusion, which would expose them to natural inoculation. Inoculations of 60 protruding Cranberry plum stigmas with *S. laxa* and the same number with *S. fructicola* produced 100% blossom blight in each case. Equal numbers of Oriole peach stigma inoculations yielded 27 and 40% blossom blight with *S. laxa* and *S. fructicola*, respectively. These high percentages suggest that protruding stigmas are important infection courts for blossom blight following mild winters.

Substances released from floral parts affect spore germination.—To determine whether the susceptibility of the different floral organs is related to the effect on spore germination of substances that may leach out of these organs, the leachates from single floral parts were used as a medium for spore germination. Blossoms were taken from Mission and Drake almond; Vedette and Oriole peach; Royal, Smyrna, and Hersey Moorpark apricot; and Emilie and Cranberry plum. Uninjured stigmas, petals, floral tubes, and anthers with closed pollen sacs from closed and open blossoms were removed, and each was placed on a drop of sterile distilled water on a clean slide and removed after one hour. To determine the effect of the nectar, one drop of distilled water was placed inside the floral tube with an elongated-tip eye dropper, and the liquid removed after one hour. To each water drop with the leachate was added a drop of spore suspension containing about 1000 spores. Incubation was for 24 hours. Eight tests were made with each floral part. Spores in distilled water served as checks.

Fig. 2.—Peach blossom with stigma protruding beyond opening. Such flowers are highly susceptible to infection and blight from both brown-rot pathogens.

A typical result from the tests with floral parts of Drake almond (Table 6) shows that leachates from the petals and floral tubes of closed blossoms and from the petals of open blossoms provided stimulus to spore germination. There were no distinct differences between *S. laxa* and *S. fructicola*. Germ-tube length was invariably greater in water drops exposed to stigmas, anthers, or the insides of floral tubes of open blossoms, than those exposed to other floral parts. Differences between hosts were not found.

The stimulation of spore germination and germ-tube elongation by the pollen-sac leachates suggested the possibility of similar stimulation from pollen. Examination of petals after the pollen sacs have opened showed the petals covered with pollen. Spray inoculations with *S. fructicola* spores on pollen-laden petals resulted in higher spore germination and longer germ-tube length than nonpollen-laden petals. Dusting the petals with pollen produced more infections per unit area than petals not dusted with pollen.

Blossom infection in the orchard.—No information on blossom inoculation of almond was found in the literature, and no comparative pathogenicity tests have been conducted with *S. laxa* and *S. fructicola* on various Prunus species.

In 1952 and 1953, field inoculations were made on New Castle and Royal apricots, Drake and Nonpareil almonds, Santa Rosa plum, Quetta nectarine, and Walton and Fay Elberta peaches, by placing on the floral parts 1-mm discs cut from the advancing edge of 2-day-old PDA plate cultures of the brown-rot organisms. Older cultures of *S. laxa* cannot be used, because growth of the pathogen is not normal, apparently being inhibited by some staling product. The inoculated blossoms were immediately covered with waxed paper bags, which in the later work were atomized inside with distilled water and contained a saturated mass of cotton. These bags were removed after 24 hours, and the number of blighted blossoms was determined a month later.

a) *Only stigma inoculations resulted in blossom blight under field conditions.*—The comparative ability of the two brown-rot pathogens to produce blossom blight in the field was tested during the blossoming seasons of 1952 and 1953. Inoculations were made on the floral tubes and petals of unopened blossoms and on the stigmas, filaments, petals, and anthers of open blossoms. Only inoculation of the stigmas produced blossom blight, as follows: For *S. laxa*, 30% on Drake almond, 53% on New Castle apricot, 20% on Royal apricot; for *S. fructicola*, 5% on Royal apricot, and 5% on Fay Elberta peach. Twig invasion was observed only on blossoms blighted by *S. laxa*. Apricot developed much more blossom blight from *S. laxa* (20–53%) than from *S. fructicola* (5%). Neither pathogen infected Nonpareil almond, Santa Rosa plum, or Walton peach.

Table 6.—Percentage spore germination and length of germ tube of *Sclerotinia* spores placed in drops of water in contact with the designated floral parts of Drake almond.

Blossom stage and floral part	*S. laxa*		*S. fructicola*	
	% germination	Germ tube length [a]	% germination	Germ tube length [a]
Closed				
Petal	73.7	–	69.2	–
Floral tube	59.0	–	74.0	–
Open				
Petal (adaxial)	54.0	–	81.4	–
Stigma	56.7	+	74.7	+
Anther	69.9	+	81.4	+
Floral tube (inside)	82.5	+	84.7	+
Check	36.1	–	52.2	–

[a] – means germ tube less than 90 μ long; + means germ tube 300 μ long, or longer.

During 1953 spores produced on Emperor grapes were collected and applied on the stigma of Bing cherry and apple blossoms. The inoculations were on April 1; it rained on April 8. Data were taken on April 19. Stigma inoculations with spores failed to produce visible infection on apple, but on Bing cherry the fungus entered the ovary and pedicel within the 19-day incubation period. Of the 20 stigmas inoculated with each pathogen before the pollen sacs burst, *S. laxa* produced 13 necrotic ovaries and 5 necrotic pedicels. In 20 inoculations made when the pollen sacs were open *S. laxa* produced only 2 necrotic ovaries and *S. fructicola* only 1, and neither fungus progressed into the pedicel.

b) *Most susceptible parts under natural conditions.*—Natural infections and blossom blight in the field were observed over a 6-year period. On almond, *S. laxa* infections were common on closed and open petals, but in most cases the infected petals dehisced before the pathogen entered the floral tube. Of infections that entered the floral tube, many failed to reach the peduncle and cause blossom blight. Anther infections were quite common, as were floral tube infections leading from the filament, a number of which were found to reach the peduncle. In each severe blossom blight season, stigma infections were the most common. Prior to blossom blight the fungus was found to have completely penetrated the ovary. With movement into the peduncle, a sudden withering of the blossom was noted. This explains a common experience of growers—that the blossoms appear healthy one day, and are wilted the next. In 1959, there was no precipitation during blossoming of the Drake variety, but heavy dews were present. The result was heavy infections in certain areas where the inoculum potential was high. Again, the infections entered through the stigma, with no evidence of floral blight from petal or filament infection.

In observations of peach varieties, during two epidemic blossom blight years, *S. fructicola* was found to be the chief pathogen. In all blossom blights examined, the fungus attacked the anthers and penetrated the filament, floral tube, and peduncle. Numerous questionable cases of stigma infections failed to show that *S. fructicola* was present.—Department of Plant Pathology, University of California, Davis.

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RHIZOPUS ROT OF PEACHES AS AFFECTED BY POSTHARVEST TEMPERATURE AND MOISTURE

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SUMMARY

Investigation was made of Rhizopus rot of peaches at 75°F as affected by previous holding of the fruit under various moisture conditions at 40° or 32°. Exposure of *Rhizopus* spores on fruit for 5 days at 40° or 32° did not affect their ability to produce decay of other peaches at 75°. Peaches held 3 or 5 days at 40° or 32° usually developed less Rhizopus rot after transfer to 75° than peaches held only 1 day at the low temperatures or peaches held constantly at 75°. The moisture conditions surrounding the fruit immediately before or after inoculation had more effect on development of decay at 75° than holding temperature of the fruit before transfer to 75°.

Introduction.—Rhizopus rot, caused by *Rhizopus stolinifer* (Fr.) Lind., usually considered less important as a postharvest decay of peaches than brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is the principal postharvest decay in certain areas of the United States. It differs from brown rot in that (a) infection usually occurs during postharvest handling, whereas brown rot usually occurs in the orchard, and (b) *Rhizopus* initially requires a wound to enter the fruit, whereas the brown rot organism may penetrate the unbroken skin. Reduction of Rhizopus rot, therefore, is limited to postharvest treatments or handling practices. Studies conducted to reduce this decay have been concerned chiefly with chemical treatments (2, 3, 4, 6, 7, 9). Under most commercial operations reduction of Rhizopus rot has usually been limited to holding peaches at low temperatures. Low temperatures generally do not kill pathogens, but do retard their growth while the produce is at low temperatures. Nevertheless, it is believed that peaches held at low temperatures develop less Rhizopus rot after transfer to higher temperatures than similar fruit not subjected to the low temperatures. This effect has been observed in this laboratory since 1951.

The present report is concerned with the effects of temperature and moisture conditions on Rhizopus rot of peaches. The data show: (a) the effect of temperatures on the virulence of *Rhizopus* spores, (b) the effect of postharvest temperatures on the peaches as related to Rhizopus rot, and (c) the effect of drying of peach wounds before or after inoculation on the development of Rhizopus rot.

Methods.—Commercially harvested peaches were obtained from wholesale houses in Washington, D. C., usually within 24 hours of being picked. Before the start of an experiment the fruit was sorted for uniform ripeness and size, and freedom from decay, cuts, or other blemishes.

To obtain a consistently high percentage of infection, peaches were inoculated with a spore suspension of *Rhizopus stolonifer*. Spores obtained from infected peaches were suspended in beef-peptone broth with enough Tween 20 (poly-oxyethylene sorbitan monolaurate) added to give a concentration of 0.1%. The concentration was 70–80 spores per low-power field of a microscope. Peaches were wounded by making a shallow cut, resembling a fingernail injury, on each cheek in 1954 and on one cheek in 1957. The fruit was inoculated by dipping the wounded cheek or cheeks into the spore suspension. In 1954 the inoculated peaches were allowed to dry and then were wrapped in copper-impregnated paper (Hartman wraps). In 1957 they were left unwrapped.

In 1954 each treatment in each replication consisted of 50 peaches, and decay readings were made after the peaches were at 75°F for 3 or 6 days. In 1957 only 15–20 peaches were used per replication in each treatment, and decay readings were made after the peaches were at 75°F for 2, 4, and 6 days. At each reading all decayed fruit were counted and discarded, and the cumulative decay at each reading date was recorded. Decay readings refer to decay in the artificially wounded and other areas of the fruit.

Experiments and results.—*Virulence of Rhizopus spores as affected by holding temperatures.*—Peaches covered with sporulating *Rhizopus* were held 5 days at 75°, 40°, or 32°F before spores were collected for inoculation. Freshly wounded fruit was then inoculated with spores from the respective temperatures and placed immediately at 75°. After 3 or 6 days at 75° the percentage of Rhizopus rot of peaches inoculated was about the same for the spores from the respective temperatures (Table 1). These data show that exposure of the spores to 40° or 32° for 5 days did not

Table 1.—Decay of peaches inoculated with *Rhizopus stolonifer* spores previously held 5 days at different temperatures.[a]

Holding temperature of spores (°F)	Peaches with Rhizopus rot (%) after indicated periods at 75°F	
	3 days	6 days
75	93	97
40	94	96
32	85	90

[a] Average of 4 replicates of 50 peaches each.

affect the virulence of *Rhizopus*.

Effect on Rhizopus rot of holding peaches at low temperature after inoculation.—Peaches were inoculated immediately after wounding and then held for 0, 1, 3, or 5 days at 40° or 32°F before being transferred to 75°. Peaches in some lots were wrapped in copper-impregnated paper (Hartman wraps). Other lots were not wrapped.

Wrapped fruit held at low temperatures for 1 day immediately after inoculation had slightly less decay after 3 or 6 days at 75°F than fruit held constantly at 75° (Table 2). Fruit held 5 days at 40° or 32° developed less decay after 3 days or 6 days at 75° than fruit held 1 day at the low temperatures or constantly at 75°. Unwrapped fruit held 1 day at 40° or 32° had considerably less decay after 2 days at 75° than fruit held constantly at 75°. Fruit held 3 or 5 days at 40° also had substantially less decay at the later readings. Unwrapped fruit held for 3 or 5 days at 40° or 32° had less decay at each reading period at 75° than fruit held at low temperatures for only 1 day and fruit held constantly at 75°.

Effect on Rhizopus rot of a delay between wounding and inoculation.—Wounded peaches were placed at 75°F for 1 day, or at 40° or 32° for 1, 3, or 5 days before being inoculated (delayed inoculation) (Table 2). Upon inoculation, some lots were placed immediately at 75° and others were held at 40° or 32° for 1 or 5 days before transfer to 75°. Decay on these fruit was compared with that on fruit placed immediately at 75° after being wounded and inoculated.

Peaches with inoculation delayed 1 day developed considerably less decay at 75°F than peaches inoculated immediately after being wounded (Table 2). The temperature during the 1-day delay period before inoculation had little influence on the development of decay. Wrapped fruit with inoculation delayed 5 days developed more decay than similar lots with inoculation delayed 1 day. In the unwrapped fruit, particularly after 4 and 6 days at 75°, there were relatively slight differences in percentage of decay among fruit held 1, 3, or 5 days between wounding and inoculation. Wrapping the fruit apparently had an effect on the wounds that favored decay when fruit was held 5 days at low temperatures before inoculation. Peaches receiving an additional low-temperature holding following delayed inoculation developed about the same percentage of decay as peaches without the added low-temperature holding period (Table 2).

Effect of humidity on development of Rhizopus rot.—Peaches were inoculated immediately after wounding and then placed in (a) open containers, (b) containers enclosed in polyethylene bags perforated with

Table 2.—Effect of delay of inoculation after wounding and various holding temperatures after inoculation on development of Rhizopus rot of peaches at 75°F.

Delay period between wounding and inoculation		Holding period after inoculation		Peaches with Rhizopus rot (%) after indicated periods at 75°F				
				Wrapped[a]		Unwrapped[b]		
Days	Temp. (°F)	Days	Temp. (°F)	3 days	6 days	2 days	4 days	6 days
0		0		93	97	82	90	90
		1	40	82	88	40	57	63
		1	32	83	88	38	80	86
		3	40			20	25	48
		3	32			5	36	58
		5	40	59	74	18	37	43
		5	32	70	84	12	25	38
1	75	0		10	24	3	23	43
	75	1	40	7	16			
	40	0		9	23	12	34	41
	40	1	40	5	25			
	32	0		6	25	7	32	48
	32	1	32	6	16			
3	40	0				3	21	25
	32	0				12	42	58
5	40	0		32	49	10	22	32
	40	5	40	17	61			
	32	0		29	42	19	38	49
	32	5	32	11	45			

[a] Average of 4 replicates of 45–50 peaches wrapped in copper-impregnated paper (Hartman wraps).
[b] 7 replicates of 15–20 unwrapped peaches.

Table 3.—Effect of type of cover,[a] temperature, and duration of holding period on Rhizopus rot of peaches inoculated immediately after wounding.

Temperature (°F) and type of cover after inoculation	Days before cover removed and fruit placed at 75°F	Peaches with Rhizopus rot (%) after indicated periods at 75°F		
		2 days	4 days	6 days
75 none		82	90	90
polyethylene[b]	1	73	85	87
polyethylene + wet cotton[b]	1	92	98	98
40 none	1	40	57	63
polyethylene	1	53	87	95
polyethylene + wet cotton	1	73	90	90
none	3	20	35	48
polyethylene	3	30	77	80
polyethylene + wet cotton	3	57	83	88
none	5	18	37	43
polyethylene	5	35	61	75
polyethylene + wet cotton	5	75	87	92
32 none	1	38	80	86
polyethylene	1	48	88	90
polyethylene + wet cotton	1	60	78	78
none	3	5	35	58
polyethylene	3	35	82	92
polyethylene + wet cotton	3	38	80	85
none	5	12	25	38
polyethylene	5	25	58	67
polyethylene + wet cotton	5	34	77	84

[a] Containers covered with polyethylene; wounds on fruit covered with wet cotton.
[b] Fruit covered 1 day at 75°F.

four ¼-in. holes, and (c) containers enclosed in polyethylene bags perforated with four ¼-in. holes with the wounded area of the fruit covered with wet cotton. Some lots were placed immediately at 75° F, whereas others were placed at 40° or 32° for 1, 3, or 5 days before being transferred to 75°. Covers were removed after 1 day from lots placed immediately at 75°, and from other lots at the time they were placed at 75°.

Peaches held constantly at 75°F and covered only during the first day had at each reading about the same percentage of decay as fruit in open containers (Table 3). Peaches in open containers held at 40° or 32° developed less decay after various periods at 75° than similar fruit held constantly at 75°. Peaches held at 40 or 32° for 3 or 5 days developed less decay at 75° than peaches held only 1 day before transfer. Fruit in containers covered with polyethylene or in covered containers with wounds covered with cotton usually developed more decay than fruit in open containers previously held at the same temperature. Fruit covered with polyethylene and held at low temperatures usually developed almost as much decay after 4 or 6 days at 75° as similar fruit held constantly at 75°. Fruit held at low temperature with wet cotton over the wounds often developed more decay at 75° than fruit in containers covered with polyethylene.

The effect of drying wounded areas before inoculation was also tested. Fruit was wounded and placed in polyethylene-covered or open containers at 75°, 40°, or 32°F for 1 day, or at 40° or 32° for 3 or 5 days. Then the coverings were removed and the fruit inoculated with *Rhizopus* spores and held at 75°. Comparisons were made between the different treatments, and with fruit inoculated immediately after wounding and held constantly at 75°.

Peaches held in polyethylene-covered containers at 75° or 40°F between wounding and inoculation usually developed more decay when uncovered and held at 75° than peaches held in open containers (Table 4). Covering the containers at 32° had little or no effect on subsequent decay at 75°. When wet cotton covered the wounds before inoculation, decay at 75° was considerably higher than that of fruit in open or polyethylene-covered containers, and approximated that of fruit inoculated immediately after wounding. After 4 or 6 days at 75° the percentage decay of fruit with the wet-cotton treatment was not appreciably affected by previous storage temperature or duration of holding before inoculation.

These data show that moisture on or surrounding the wounded areas has more effect on the development of Rhizopus rot of peaches than the temperatures at which the peaches are held.

Discussion.—Infections developing after spore inoculation constitute one of the most common methods of spread of Rhizopus decay of peaches. Any method of handling fruit that would decrease infection by *Rhizopus* spores would result in an appreciable saving to the peach industry. Several factors may influence germination of the spores and germ tube penetration into the fruit.

Earlier reports showed that peaches held 5 days at 40°F or 14 days at 31° developed less Rhizopus rot when transferred to higher temperatures than similar

Table 4.—Effect of type of cover[a] temperature and duration of holding periods on Rhizopus rot of peaches with delayed inoculation.[b]

Temperature (°F) and type of covering before inoculation[c]	Days between wounding and inoculation	Peaches with Rhizopus rot (%) after indicated periods at 75°F		
		2 days	4 days	6 days
75 none[d]	0	60	74	79
none	1	3	23	43
polyethylene	1	33	48	65
polyethylene + wet cotton	1	55	75	79
40 none	1	12	34	41
polyethylene	1	9	45	59
polyethylene + wet cotton	1	34	81	87
none	3	3	21	25
polyethylene	3	11	43	53
polyethylene + wet cotton	3	53	75	81
none	5	10	22	32
polyethylene	5	17	45	58
polyethylene + wet cotton	5	61	76	82
32 none	1	7	32	48
polyethylene	1	9	38	58
polyethylene + wet cotton	1	45	77	80
none	3	12	42	59
polyethylene	3	15	54	66
polyethylene + wet cotton	3	55	88	93
none	5	19	38	49
polyethylene	5	21	37	48
polyethylene + wet cotton	5	74	81	87

[a] Containers covered with polyethylene, wounds on fruit covered with wet cotton.
[b] Interval between wounding and inoculation.
[c] All fruit was uncovered at 75°F.
[d] Fruit inoculated immediately after wounding and placed at 75°F.

peaches held at the higher temperatures constantly (4, 5, 6). Low temperatures may have an adverse effect on the spores or the mycelium of *Rhizopus* or some effect on conditions for infection of the fruit. In the present study *Rhizopus* spores held 5 days at 40° or 32°F produced as high a percentage of decay of freshly wounded and inoculated peaches as spores not held at the low temperatures. This period of exposure to the low temperatures did not affect the germination of the spores or subsequent penetration of the fruit. Nevertheless, freshly wounded and inoculated peaches held 1–5 days at 40° or 32° often developed considerably less decay at 75° than similarly inoculated peaches held constantly at 75°. Because *Rhizopus* spores do not germinate at 40° or below, no mycelium was present in these tests. Therefore, the decay reduction associated with the low-temperature holding period of peaches in these tests appears to result from a response of the fruit rather than of the organism.

Germ tubes from *Rhizopus* spores usually cannot infect peaches except through wounds. Peaches wounded and held 1 day at 75° F or 1, 3, or 5 days at 40° or 32° before inoculation developed considerably less decay at 75° than peaches inoculated immediately after wounding and held constantly at 75°. The decrease in decay from delaying inoculation was about the same in fruit held at 75°, 40°, or 32°. Thus, decreased decay from delayed inoculation was independent of the holding temperature. Keeping the wounded areas moist increased the percentage of Rhizopus rot at 75°F, regardless of the previous holding temperature. When the wounded areas dried most rapidly before inoculation, percentage of Rhizopus decay was lowest. Decay of peaches at 75° when previously held at 75°, 40°, or 32° under conditions that would permit wound drying before inoculation was approximately the same. During commercial storage of peaches refrigerated air may be low in relative humidity and cause drying of wounds, thus creating a condition unfavorable for infection by *Rhizopus* spores. Drying of wounded areas of the fruit apparently provides resistance to the germ tubes of *Rhizopus* spores. This offers a reasonable alternative explanation of the reduction of *Rhizopus* decay of peaches that has been associated with low-temperature holding.

From these data it may be postulated that peaches hydrocooled and packed wet, or those packaged in transparent films, both methods that retard wound drying, will have more Rhizopus decay than peaches handled by methods permitting wound drying. Experimental data already presented (1), showing an increase in decay of hydrocooled peaches, support this premise.—Agricultural Marketing Service, Plant Industry Station, Beltsville, Maryland.

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THE EFFECT OF RHAMNUS PALAESTINA ON THE ORIGIN AND PERSISTENCE OF OAT CROWN RUST RACES

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SUMMARY

Isolates from 75 aecial collections from *Rhamnus palaestina* secured in winter and early spring in 1956–59 consisted of a number of oat crown rust races prevalent in similar order on oats in the main period of the disease outbreaks, March–May. The principal races parasitic on both buckthorn bushes and oats are: the 276-264-263 group and races 286, 277, and 270, all virulent on Landhafer and Santa Fe, and the race 202-203 complex, capable of attacking Bond. The results attest to the importance of *R. palaestina* in the persistence of at least some physiologic races of oat crown rust from season to season. There is evidence, however, that other media also make the perpetuation of the fungus possible. It was further ascertained that numerous oat crown rust races have originated on the buckthorn plants, 11 of them being entirely new and not heretofore described. The intensity of crown rust development on the principal and alternate hosts is not necessarily correlated. In some instances, susceptible oat varieties remained healthy in close proximity to heavily rusted buckthorns harboring virulent races. The need of environment favoring crown rust incidence is also indicated.

Introduction.—Previous study (20, 32) reported evidence indicating the importance of *Rhamnus palaestina* Boiss. as an alternate host in the life cycle and physiologic specialization of *Puccinia coronata* Cda. var. *avenae* Fraser & Ledington. These findings, the presence of extremely virulent races of the fungus, and severe annual oat crown rust outbreaks stimulated expanded research on the relation of buckthorn plants to the origin and persistence of physiologic races of oat crown rust in Israel.

Since de Bary (2) demonstrated heteroecism in *P. coronata* and ascertained the role of buckthorn bushes in the life cycle of this parasitic organism, there have been numerous investigations on the various aspects of this discovery. They are adequately reviewed by Viennot-Bourgin (31), Johnson (12), and others. Some relevant to the present studies are briefly discussed herein.

Uredinologists in various countries disagree on the significance of *Rhamnus* spp. in perpetuating and disseminating oat crown rust. Straib (30) claimed that aecia harbored on common species of buckthorn in Germany (possibly excepting *R. cathartica*) are primarily associated with crown rust on grasses. His conclusions gained additional support in Canada (12, 19). In contrast, results in the U. S., U. S. S. R., several European countries, and recently also in Canada were entirely different. Dietz (4) asserted that Rhamnus plants are media for distribution of oat crown rust. Melhus and Kent (15) diagrammed the spread of inoculum from buckthorn to oat fields. McKay (14) recommended buckthorn eradication to prevent spread of the disease in Ireland. Gorlenko (9) attempted to prove that *R. cathartica* constitutes a direct source of oat crown rust infection in the Voronesh region of the U. S. S. R. Geshele (8) maintained that oat crown rust in Siberia is invariably associated with buckthorn shrubs. Mynkevitshous (17), in Lithuania, and Mytshens (18), in Latvia, advised eradication of buckthorn as responsible for early-season infection and multiplication of oat crown rust. Green et al (10) and Johnson et al (13) emphasized that most of the aecial collections isolated from *R. cathartica* belonged to the variety of *avenae*. Johnson et al (13) also stressed the agreement between the results of the rust survey based on collections made on oat varieties and buckthorn plants.

The possible relation of *Rhamnus* spp. to the origin of new physiologic races of *P. coronata* var. *avenae* was indicated in a number of publications. Murphy (16) assumed that the continuous population shifting of oat crown rust races is largely due to hybridization and segregation of the fungus on the alternate host. Johnson (12) believed that the sexual stage of *P. coronata* on Rhamnus functions in the same fashion as stem rust on Berberis, and concluded: "The question of the relationship of buckthorn . . . to the continuous succession of races of oat-crown rust merits more in-

vestigation than it has received hitherto." Stakman and Rodenhiser (29) postulated that Rhamnus eradication is desirable for the development oat varieties permanently resistant to crown rust.

The present investigation was undertaken to elucidate the role of the abundant Rhamnus vegetation in Israel: 1) in initiating early-season oat crown rust incidence, 2) in perpetuating the fungus up to the main season of crown rust outbreaks in March–May, 3) in the origin of new physiologic races of the parasitic organism.

The Rhamnus vegetation of Israel.—The following 4 Rhamnus species are present in this country (5): *R. palaestina* Boiss., *R. Alaternus* L., *R. punctata* Boiss., and *R. disperma* Ehrenb. Pycnia and aecia completing the oat crown rust life cycle have been found here only on *R. palaestina* and *R. Alaternus* (20, 32). Similar observations on the latter species have been reported from the U. S. S. R. (31). Our research deals mainly with *R. palaestina*, endemic in Israel and Syria and considered as an East Mediterranean species (6). It belongs to the most common and characteristic shrub elements of the native Mediterranean vegetation, penetrating occasionally to arid regions. The bushes are deciduous; some types of this species begin to form new foliage early in December, but new leaves ordinarily appear in January–March.

Relation of R. palaestina to early-season oat crown rust incidence.—In seasonal development of oat crown rust, 2 periods are distinguishable (32). On the wild oats of *Avena sterilis* L., on volunteer plants, and on early-sown irrigated fields, uredia usually appear in appreciable amounts early in November, or even by the end of October. In some years uredia were already collected in late September. Low temperatures in December–February inhibit the spread of the disease on oats. The principal period of oat crown rust attacks starts in March, reaches a climax by mid-April, and declines in May. Telia are generally produced in May, but have been observed in April on varieties like Bond and Clinton. This is particularly true in southern Israel. Surveys made in 1955–59 prove that pycnia and aecia begin to appear on *R. palaestina* in December in Upper Galilee, Western Galilee, the hills of Samaria (half-way between Haifa and Tel-Aviv), Mount Carmel (near Haifa), and the Jerusalem area. Pycnia and aecia may develop side by side on both sides of the young leaves, on young twigs, and on all floral parts and young fruits, causing slight deformation of the affected tissue. Pycnia frequently excretes a shiny exudate; aecia are present on the young plant parts throughout the winter, being perceptible until mid-May if the rainy season ends late.

In addition, telia of Puccinia *mesnieriana* Thüm. also thrive profusely on *R. palaestina* in winter. It should be noted that pycnia and aecia of oat crown rust appear concurrently on *R. Alaternus* and *R. palaestina*. Only in 1957 aecia of *P. coronata avenae* were collected from *R. Alaternus* in November, presumably because fall rains were early that year.

Obviously, aecia making their first appearance in December cannot possibly account for the initial infection of early season occurring on oats as early as October-November, and in some instances even in September. Some other media are evidently responsible for the persistence of the inoculum inciting crown rust on oats early in the season.

Persistence of inoculum inciting oat crown rust in spring.—The successive occurrence of aecia on *R. palaestina* in winter and early spring, and of main crown rust outbreaks on oats in March-May suggested an investigation of whether and how much an inoculum on buckthorn is responsible for the upsurge in spring. To answer this question, the racial identity of aecial isolates from 75 collections secured from *R. palaestina* in 1956–59 has been studied and compared with the race population in 106 collections obtained from oats in spring.

Materials and methods.—Aeciospores were collected for oat inoculation by Peturson's method (19). A rusted buckthorn leaf carrying a single cluster of aecial cups was inserted into a sheet of moist filter paper appressed to the inside of a Petri dish cover. Subsequently, the lid was replaced over the bottom of the dish in which a microscope slide was kept. Spores began discharge within several hours. The deposited spores were transferred to the first leaf of 8–10-day-old seedlings of the extremely susceptible oat variety Fulghum. The inoculation technique and the procedure followed during incubation were described by Stakman et al (28). At the end of 10–13 days uredia began to appear. Unipustular isolates from the infected seedlings were increased on a new series of Fulghum seedlings and then used in race identification tests. The racial identity of isolates thus secured was determined with the aid of diagnostic keys (21, 22, 23, 24, 25, 26) on the basis of reactions produced on the seedlings of standard differential varieties (26). Similar methods were resorted to in identifying monopustular isolates derived from oats rusted in the fields; only one isolate from each collection was identified.

In trials designed to compare the racial characteristics of single aecial cups belonging to the same cluster, the particular cup was transferred with dissecting needles to moistened and debloomed leaves of Fulghum seedlings. Only very young cups were selected.

Results.—Table 1 illustrates the composition of race populations parasitizing *R. palaestina* and oats in spring. Not included are new races isolated from the principal or alternate host. It should be mentioned that 4 aecial isolates were innocuous on Fulghum seedlings, though their viability was established by spore germination tests. They, too, are not in Table 1.

It is realized that information gained by testing a limited number of crown rust collections does not warrant general conclusions. Nevertheless, several interesting implications are inferred from the tabulated results. The composition of race populations on *R. palaestina* and oats seems to follow certain patterns exhibiting resemblance in some important points. The so-called "Landhafer group" (25) of races—276-264-263—is distinctly prominent on the principal and al-

Table 1.—Identity and prevalence of physiologic races of oat crown rust isolated in 1956–59.[a]

Physiologic race	Number of times each race was found in: Aecial collections	Uredial collections
Group 276-264-263	23	43
Group 202-203	11	14
286	6	13
270	5	12
Group 216-217[b]	–	9
277	5	6
201	–	2
206	1	–
207	1	–
209	–	1
210	–	1
211	3	1
212	1	–
213	–	2
220	1	–
221	4	–
224	2	1
249	1	–
279	–	1

[a] New physiologic races hitherto unknown and discovered on oats and *R. palaestina* are not included. They are described elsewhere (33).

[b] This group was absent in uredial collections in 1958–59.

ternate hosts. It is noteworthy that race 264 was originally discovered in Israel and predominated here in 1953–55 also. The agreement in race situation on buckthorn and on oats is manifested also by races 202-203, 286, 270, and 277. Race 202-203, ranking first in the U. S. until recently (25) has been second in order of prevalence in the isolates secured from both *R. palaestina* and oats. Races 286, 270, and 277, and the race group 276-264-263 have in common the capability of attacking Landhafer and Santa Fe. In view of the serious concern aroused in the U. S. by the recent increase in prevalence and distribution of races virulent on Landhafer (24, 25), their predominance on buckthorn may be particularly significant. On the other hand, it should be stressed that the "Victoria race" group, 216-217, amounting to 10.5% of the uredial isolates in 1956–58 (but absent in 1959 collections) has not been detected on *R. palaestina* so far.

The analyzed data seem to suggest that *R. palaestina* is responsible to a large extent for perpetuation of some of the extremely virulent and very prevalent crown rust races attacking oat crops in spring. The role of the early-season crown rust incidence in furnishing inoculum for outbreaks in March–May has not yet been investigated.

Our field observations have further shown that favorable environmental conditions also are indispensable to oat crown rust epiphytotics. Uniform oat-testing field nurseries planted at Yiftah (Upper Galilee) in 1955 were only slightly affected by crown rust although the nurseries contained a number of very susceptible varieties and were near *R. palaestina* bushes. In the winter of 1958–59 a nursery was found to be 50–60 m from 2 heavily rusted buckthorn shrubs harboring aecia of race 276 group, and races 286, 224, and 202. All cultivated varieties, and wild oats of *A. sterilis*, growing abundantly 2–3 m from the rusted buckthorns, remained practically rust-free though the susceptibility of *A. sterilis* to the mentioned races was proved. Our observations corroborate Gorlenko (9), who reported that profusely rusted bushes of *R. cathartica* were not sufficient to develop epiphytotics on oats in the Voronesh region in U. S. S. R. in the absence of suitable climatic conditions.

Relation of R. palaestina to the origin of oat crown rust races.—The idea that some Rhamnus species may play the same role in the rise of physiologic races of oat crown rust as Berberis in stem rust was conceived by several investigators (12, 16, 29), and supporting evidence has been offered (16). Consequently, certain buckthorn species are considered at least partially responsible for shifts in the populations of oat crown rust races.

The effect of *R. palaestina* on the origin of physiologic races of *P. coronata* var. *avenae* has been studied, and the results are presented.

Results.—Race identification tests conducted with aecial material revealed in 75 collections studied the existence of 11 entirely new races not hitherto reported. The diagnostic reactions induced by these races on seedlings of standard differential oat varieties are recorded in Table 2.

Those results attesting to the significance of *R. palaestina* in the creation of oat crown rust races can be supplemented by consulting Table 1. Races 206, 207, 212, 220, 221, and 249 have thus far been found here only on *R. palaestina*.

Stakman et al (27) called attention to the diversity of stem rust races in aecial collections in contrast with less heterogeneous race populations on cereals in areas where barberries rarely become rusted. Similar conclusions can be made with respect to oat crown rust. The data in Tables 1 and 2 show that 24 races were isolated from 75 aecial collections, about 1 race from every 3 collections. In the uredial material from 106 collections provided from oats, 13 races could be isolated—a 1:8 ratio.

Actually, those computations do not fully reflect the variability of race populations on *R. palaestina*, because of the shortcomings of the isolation procedure. As previously stated, the inoculum employed was derived from single aecium clusters, which are of a complex genetic makeup and comprise more than 1 race. This could be demonstrated by using single aecial cups as a source of inoculum following the method described above. Five aecial clusters were investigated. The results (summarized in Table 3) seem to prove that each aecial cluster consists of a number of physiologic races. Some preliminary observations indicate that a single aecial cup may also contain more than 1 race, as was shown by Cotter (3) with *P. graminis*.

Nevertheless, in the heterogeneous race composition of the aecial collections some common features and trends became pronounced. For instance, Tables 1 and 2 relate that 71% of races isolated from *R. palaestina*

Table 2.—Reaction of standard differential oat varieties to 11 new physiologic races of oat crown rust discovered on *Rhamnus palaestina.*

Race No.[b]	Reaction of indicated variety[a]									
	Anthony	Victoria	Appler	Bond	Landhafer	Santa Fe	Ukraine	Trispernia	Bondvic	Saia
300	S	R	R	S	R	S	R	S	S	R
301	S	R	R	S	R	S	S	S	S	R
302	S	R	S	S	Int	R	R	S	S	R
303	S	S	S	R	R	S	R	S	S	R
304	S	R	S	S	R	S	S	S	S	R
305	S	S	S	R	S	S	R	S	S	R
306	S	R	R	R	R	R	R	S	S	R
307	S	R	R	S	R	R	R	S	S	R
308	S	R	S	S	Int	S	R	R	S	R
309	S	S	S	S	S	S	S	S	R	R
310	S	R	R	R	R	S	S	R	S	R

[a] R = resistant; S = susceptible; Int = indicates that the reaction of the variety cannot be clearly classified as resistant or susceptible.
[b] Numbers assigned by Dr. M. D. Simons.

are virulent on Bond, 50% on Santa Fe, 33.5% on Landhafer, and 17% on Victoria. It may be worth mentioning that "Victoria races" are reported to be of rare occurrence on buckthorn in the U. S. also (21).

Crown rust records taken in 1952–59 from uniform oat field nurseries located in most regions of the country seem to reflect the race situation in the aecial collections. Bond and its derivatives and Santa Fe have rusted heavily through the years in most nurseries, and Victoria and Victoria hybrids have displayed moderate resistance. Landhafer was considerably less affected by crown rust than could be anticipated from the percentage prevalence of the "Landhafer races" on buckthorn. Landhafer may possess a certain degree of field resistance to these races, as seems to be the case in the U. S. (25).

Discussion.—The foregoing results seem to indicate that the role of *R. palaestina* in oat crown rust parallels that of Berberis in stem rust. Buckthorn bushes are important at least in 2 ways: 1) they enable certain very virulent and prevalent physiologic races of *P. coronata* var. *avenae* to survive under unfavorable environmental conditions and to eventually incite serious disease outbreaks in March–May, and 2) they facilitate the creation of new physiologic races.

It is noteworthy that the most common races isolated in 1956–59—the so-called "Landhafer race group," 276-264-263 (25); the race 202-203 complex virulent on Bond; and races 286, 270, and 277—appear in a similar order of frequency on oats and on *R. palaestina.* Since races 286, 270, and 277 are virulent on Landhafer also, the predominance of "Landhafer races" on the alternate host merits particular attention. On the other hand, the "Victoria races"—group 216-217, amounting to about 8.5% of the uredial isolates—were conspicuously absent from the aecial material. Because of their abundance and wide distribution, the buckthorn plants obviously enhance the multiplication and spread of crown rust; however, aecia harbored on the alternate host from December throughout the winter cannot possibly furnish inoculum for the early-season crown rust incidence in October-November.

It was further ascertained that numerous physiologic races of oat crown rust have originated on *R. palaestina;* for example, 11 entirely new races not hitherto reported were identified from 75 aecial collections. Because new races arise continually, the race population thriving on buckthorn becomes diversified. For comparison, it may be worth mentioning that in Murphy's (16) investigations, 65 aecial collections represented 14 races. According to Simons (22), 61 aecial isolates, originating primarily on *R. cathartica* consisted of 14 races, with races 202 and 203 being most common. On the other hand, uredial collections are distinctly more homogeneous in racial makeup in the U. S. (21, 22, 23) than in Israel. Presumably, the effect of hybridization of the fungus on the alternate

Table 3.—Physiologic races of oat crown rust identified from single cups of 5 aecium clusters collected on *Rhamnus palaestina.*

Aecium cluster	Races identified
First: 5 cups infectious of 6 isolated	202, 202, 207, 276, and one new race represented by isolate 11 in Table 2.
Second: all 3 isolated cups infectious	202, 263, 276
Third: 4 cups infectious of 6	224, 263, 276, and one new race represented by isolate 10 in Table 2
Fourth: 4 cups infectious of 5	202, 211, 276, 276–286[a]
Fifth: all 5 isolated cups infectious	203, 224, 263, 263, 286

[a] Racial identity of the indicated isolates could not be clearly determined since Bond showed reactions of both resistant and susceptible class.

host, as expressed by the degree of variability of the racial composition in the uredial stage, becomes more pronounced in a small country like Israel with an abundant buckthorn flora.

The suggestion that the evolutionary development of oat rusts has taken place in this region (32, 34) is corroborated by the similarity in races population on cultivated oats, wild oats indigenous to the Mediterranean area, and on the East-Mediterranean buckthorn species *R. palaestina*. An additional substantiation of this evolution concept may be gained from the annually observed coexistence of the micro-type rust fungus *P. mesnieriana*, with pycnia and aecia of the eu-typic, heteroecious life cycle of *P. coronata* var. *avenae*. The correlation of these organisms was first pointed out by Dietel (1) and also discussed by Viennot-Bourgin (31). Dietel (11) postulated that the micro-type fungus constitutes a primitive stage in evolution that ultimately gave rise to more advanced, highly specialized long cycle forms. Gäumann (7), in contrast, assumed that the micro-type is of recent origin and represents "the end of a retrogressive evolutionary sequence." Our attempts to investigate this problem experimentally have been seriously handicapped by the prolonged dormancy of teliospores of both rusts.

The data reported seem to justify recommending the eradication of buckthorn species associated with the life cycle of oat crown rust, a measure not economically feasible in Israel.—The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel.

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PHYTOPATHOLOGICAL NOTES

FERTILIZATION OF PYCNIA WITH UREDIOSPORES AND AECIOSPORES IN PUCCINIA GRAMINIS

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Cooperative investigations between the Plant Pest Control Division, ARS, U. S. D. A., and the Minnesota Agricultural Experiment Station. Paper No. 4295, Scientific Journal Series, Minnesota Agricultural Experiment Station.
Plant Pathologist, Plant Pest Control Division, ARS, U. S. Department of Agriculture. The author is indebted to Dr. E. C. Stakman, who suggested this line of investigation.

Since Craigie (3) discovered the function of pycnia, in 1927, it has been demonstrated repeatedly that aecia develop when pycniospores of one sex are transferred to flexuous hyphae of the other sex or when the mycelium of a pycnium fuses with the mycelium of another pycnium (2) or a uredium (1). No one, however, has reported experiments on urediospores or aeciospores as possible spermatizing agents.

Experiments were made with *Puccinia graminis* Pers. vars. *tritici* (Eriks. & E. Henn.) Shear, Ball, Jacks., & Stakman, and *secalis* (Eriks. & E. Henn.) Seym. Potted barberry seedlings about 8–12 in. tall were inoculated in the greenhouse with telial material from the upper Mississippi Valley. As soon as pycnia appeared, plants with no more than 6 pycnia were placed under bell jars to avoid contamination by insects. The pycnia on such plants were widely separated.

Since aecia on unprotected plants normally form about 1 week after pycnia appear, the pycnia that developed under bell jars were considered to be haploid when there was no evidence of aecial formation at the end of 10 days. Either freshly collected urediospores or aeciospores were mixed with the pycnial nectar on the surface of the apparently haploid pycnia. At least 1 pycnium on each plant was kept as an untreated check, and in no case did aecia form beneath the untreated pycnia.

Table 1.—The results of applying urediospores and aeciospores of Puccinia graminis to pycnia of the same species on barberry.

Fertilizing agent	Variety of pycnia and number of aecial clusters			
	var. *tritici*		var. *secalis*	
	Trials	Success	Trials	Success
Aeciospores				
var. *tritici*	35	13	6	2
var. *secalis*	6	0	39	11
Urediospores				
var. *tritici*	82	16	22	2
var. *secalis*	1	0	16	2
Totals	124	29	83	17

It was evident that both urediospores and aeciospores could induce the development of aecia; they were produced in 20 of 121 trials with urediospores and in 26 of 86 trials with aeciospores (46 successes in 207 trials). These results suggest that aeciospores are more efficient than urediospores as spermatizing agents, for the proportion of successful trials was greater. The time required for formation of aecia was about the same in both cases—5–12 days.

It is apparent also that the spermatizing of pycnia was most successful with urediospores and aeciospores from the same rust variety (Table 1), although *tritici* urediospores or aeciospores applied to *secalis* pycnia induced the development of aecia in a limited number of trials.

The racial identity of 46 of the aecial cultures, involving 22 crosses (including 1 intervarietal cross), was determined with the standard stem rust differential varieties (4). In all, 17 different races of *P. graminis* var. *tritici* and 3 of *P. graminis* var. *secalis* were identified. One of the races of *tritici* obtained had not previously been identified but is now known as race 159.

This is the first report that urediospores and aeciospores can be used as spermatizing agents in studies of selfing and crossing in the rusts. Here is another method by which nuclear association may be made in rust fungi. The results were similar to those obtained when pycniospores were used as spermatizing agents.

The method may be of value in studying rust genetics when particular combinations of genetic material are wanted but when pycnia of the desired races are not available at the same time. It introduces a new method of pairing nuclei in rust fungi. This will permit certain genetic analyses of individual nuclei without meiosis and thus provide a useful tool for studying direct relationships between dicaryotic nuclei.

The work was recently confirmed by Roy D. Wilcoxson and Wiley N. Garrett, at Minnesota, whose results will be published soon.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

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A METHOD FOR SCREENING ALFALFA PLANTS FOR RESISTANCE TO PSEUDOPEZIZA MEDICAGINIS

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Common leaf spot, caused by *Pseudopeziza medicaginis* (Lib.) Sacc., is one of the major foliar diseases of alfalfa (*Medicago sativa* L.) in humid areas. Greenhouse techniques are needed for testing large populations of plants for disease resistance. Greenhouse testing has advantages over field testing in that severe epidemics are assured, less time and effort are needed per unit plant, and evaluations are not complicated by symptoms caused by other pathogens.

Previously, test plants in greenhouse moist chambers were artificially inoculated by suspending naturally infected alfalfa leaves bearing apothecia of *P. medicaginis* over them (1, 3). This method is unsatisfactory because naturally infected leaves are often contaminated with other foliar pathogens, and obtaining leaves with mature apothecia of *P. medicaginis* is sometimes difficult. Therefore, a method was developed for culturing the pathogen, inoculating plants with pure cultures of the fungus, and evaluating plants for resistance.

P. medicaginis was isolated from alfalfa leaves in a manner similar to that described by Jones (2). Leaves bearing apothecia were fastened to the underside of Petri plate covers over water agar at 20°C. Ascospores were usually discharged within 24 hours. Masses of ascospores on small agar discs were then transferred to oatmeal agar slants in test tubes. The slants were usually covered with colonies of the fungus within 4 weeks. The rapidity of growth was proportional to the number of ascospores transferred.

Fungus inoculum was increased by aseptically comminuting cultures from the oatmeal agar slants in sterile distilled water and pouring 1 ml of the suspension over the surface of 2% oatmeal agar in each Petri plate. Cultures were incubated at 20°C. In 3–4 weeks numerous colonies grew on the agar surface and were discharging ascospores. The culture plates were then inverted over alfalfa plants in a moist chamber for 48 hours at a temperature not exceeding 21°C.

The moist chamber consisted of a wooden frame covered with transparent sheet plastic, with a slatted floor to support the plant containers. A shallow pan containing water warmed slightly by an incandescent lamp beneath it was fitted inside the frame beneath the floor.

The plants to be screened were spaced 1 in. apart in flats. Some plants were inoculated as soon as the unifoliate leaves unfolded, but infection was more uniform when 1 to several trifoliolate leaves were present. The plants were removed from the moist chamber and incubated on a greenhouse bench at about 21°C. Lesions appeared on the leaves of susceptible plants in 14–20 days.

The reactions of the plants were rated according to type of lesion and amount of chlorosis. Symptomless plants or those with pinpoint spots were rated highly resistant. If leaves had lesions of less than 1 mm diameter in which no apothecia developed, the plants were rated moderately resistant. Plants with leaves bearing lesions containing apothecia and plants on which considerable chlorosis developed were rated susceptible.

Susceptible plants were discarded and the remainder reinoculated to detect escapes. Only plants rated highly resistant were retained for use in the breeding program.

With this method, large numbers of plants from various sources were screened in the greenhouse, and 506 plants rated highly resistant were transplanted into the field together with plants of a known susceptible clone. In a severe field epidemic of common leaf spot that followed, fewer than 2% of the selected plants were rated susceptible, whereas all plants of the susceptible check became heavily infected.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul.

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